



# Magnetic Resonance Imaging for Prediction and Assessment of Treatment Response in Bevacizumab-Treated Recurrent Glioblastoma

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## **Abstract**

Glioblastoma is the most common primary brain tumor in adults, and it is associated with a dismal prognosis with a median survival of 15 months. Despite treatment with chemotherapy, radiation therapy and surgery, patients inevitably have disease recurrence. Bevacizumab is a monoclonal humanized antibody that inhibits vascular endothelial growth factor signaling, and it has been shown to be effective in recurrent glioblastoma with respect to prolonging progression-free survival (PFS). The use of bevacizumab and other anti-angiogenic agents in recurrent glioblastoma have created novel challenges in interpreting magnetic resonance imaging (MRI) of patients. Furthermore, since only some patients appear to have a durable benefit from bevacizumab, there is a need for imaging biomarkers that can reliably identify this subgroup of patients.

Partly due to the challenges created by anti-angiogenic agents, the Response Assessment in Neuro-Oncology (RANO) was proposed to address some of the limitations with traditional response assessment criteria. In the first part of this project (Study 1), we attempted to validate the RANO criteria by performing a comparative analysis of the RANO criteria vs. the Macdonald criteria using imaging from the phase II BRAIN trial. As we hypothesized, the RANO criteria yielded a significantly decreased PFS by identifying a subset of patients who had progression of nonenhancing tumor evident on T2-weighted imaging. Additionally, response and progression as defined by the RANO criteria correlated with subsequent overall survival (OS) in landmark analyses. While this supports the implementation of RANO criteria for response assessment in glioma clinical trials, future research will be necessary to further improve response assessment by incorporating advanced techniques such as volumetric anatomic assessment, perfusion-weighted MR (PWI-MR), diffusion-weighted MR (DWI-MR), MR spectroscopy (MRS) and positron emission tomography (PET).

Advanced imaging techniques are becoming increasingly recognized for their ability to provide objective, non-invasive assessment of treatment response but also to serve as predictive and prognostic biomarkers allowing for stratification of patient subgroups with better treatment outcome. In the second part of the project, we attempted to perform volumetric analysis of tumor size based on conventional MRI, as well as a histogram analysis of apparent diffusion coefficients (ADC) derived from diffusion-weighted MRI, to evaluate imaging parameters as predictors for PFS and OS in a single institution database of recurrent glioblastoma patients initiated on bevacizumab. Volumetric percentage change and absolute early post-treatment volume (3-6 weeks after initiation) of enhancing tumor can stratify survival for patients with recurrent glioblastoma receiving bevacizumab therapy (Study 2<sup>a</sup>). ADC histogram analysis using a multi-component curve-fitting technique within both enhancing and nonenhancing components of tumor prior to the initiation of bevacizumab can also be used to stratify OS in recurrent glioblastoma patients (Study 3<sup>a</sup>). While prospective studies are necessary to validate findings, future studies will increasingly incorporate multiparametric approaches to elucidate biomarkers that combine the value of conventional MRI with advanced techniques such as DWI-MR, PWI-MR, MRS and PET to obtain better predictors for PFS and OS in recurrent glioblastoma.

<sup>a</sup>Publications resulting from the work described in this thesis

The study described as Study 2 in this thesis has been published in *Cancer*. Reference: Huang RY<sup>#</sup>, Rahman R<sup>#</sup>, Hamdan A, et al. Recurrent glioblastoma: Volumetric assessment and stratification of patient survival with early posttreatment magnetic resonance imaging in patients treated with bevacizumab. *Cancer*. 2013. doi:10.1002/cncr.28210 [<sup>#</sup>co-first authors].

A manuscript detailing Study 3 has been accepted for publication in the *Journal of Neuro-Oncology*. Reference: Rahman R, Hamdan A, Zweifler R, Jiang H, Norden AD, Reardon DA, Mukundun S, Wen PY, Huang RY. ADC Histogram Analysis of Apparent Diffusion Coefficient within Enhancing and Nonenhancing Tumor Volumes in Recurrent Glioblastoma Patients Treated with Bevacizumab. Accepted for publication in the *Journal of Neuro-Oncology*.

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## **Abbreviation Key**

**VEGF:** vascular endothelial growth factor

**RR:** response rate (radiological)

**PFS-6:** progression-free survival at 6 months

**OS:** overall survival

**PFS:** progression-free survival

**MRI:** magnetic resonance imaging

**FLAIR or T2/FLAIR:** fluid attenuated inversion recovery, T2-weighted

**RANO:** Response Assessment in Neuro-Oncology

**RECIST:** Response Evaluation Criteria in Solid Tumors

**MRS:** magnetic resonance spectroscopy

**PWI-MR:** perfusion-weighted magnetic resonance imaging

**PET:** positron emission tomography

**DWI-MR:** diffusion-weighted magnetic resonance imaging

**ADC:** apparent diffusion coefficient

**fDM:** functional diffusion map

**KPS:** Karnofsky performance status score

**C-index:** statistical parameter derived from concordance statistics

**T1+C:** enhancing tumor component on T1-weighted imaging

**rNTR:** ratio of nonenhancing tumor to enhancing tumor

**HR:** hazard ratio

**ADC<sub>L</sub>:** peak of low region of histogram with multi-component fitting, with ADC values < 1050

$\times 10^{-6} \text{mm}^2/\text{sec}$

**ADC<sub>M</sub>:** peak of middle two regions of histogram with multi-component fitting, composed of two components with fixed peak centers at  $1150 \times 10^{-6} \text{mm}^2/\text{sec}$  and  $1350 \times 10^{-6} \text{mm}^2/\text{sec}$

**ADC<sub>H</sub>:** peak of high region of histogram with multi-component fitting, center at  $1550 \times 10^{-6} \text{mm}^2/\text{sec}$

**IRF:** independent radiology facility

**nCBV:** normalized cerebral blood volume

# Introduction to Recurrent Glioblastoma and Its Imaging

## *Recurrent Glioblastoma: Background, Epidemiology, Treatment*

Over 20,000 of primary brain tumors are diagnosed annually in adults in the US, and almost two-thirds of these tumors are high-grade gliomas.<sup>1</sup> Within this subset, over half are given the histopathological diagnosis of glioblastoma, which represents the most aggressive and most common primary brain tumor with an incidence of 3.2 per 100,000 in the United States.<sup>2</sup>

The current standard of care for newly diagnosed glioblastoma was established by a large, multi-institutional phase III trial that demonstrated the benefit of adding temozolomide to surgery and radiation.<sup>3</sup> Despite this advancement, glioblastomas are still associated with a dismal prognosis. The median survival for patients with newly diagnosed glioblastoma is 15 months.<sup>4</sup> Given its aggressive, infiltrative nature, glioblastoma inevitably progresses through initial therapy. At the time of disease recurrence, there are limited options available for patients. For recurrent glioblastoma, the median progression-free survival is 9 months,<sup>5</sup> and the median survival of patients is 25 to 40 weeks.<sup>6–8</sup>

Prior to recent advances, the use of chemotherapeutic agents such as procarbazine and nitrosoureas were the standard of care for recurrent glioblastoma.<sup>9,10</sup> Based upon two recent phase III trials of nitrosourea monotherapy in recurrent glioblastoma,<sup>11,12</sup> these agents have been shown to have meager response rates (RR) of less than 10%. Single-agent irinotecan subsequently became a common agent to employ at recurrence of glioblastoma. With several phase II studies indicating a RR of less than 15%, this topoisomerase I inhibitor was also ineffective for the vast majority of patients.<sup>13–15</sup>

In light of recent advances in our understanding of the underlying molecular characteristics of glioblastoma, targeted therapy has been pursued to improve outcomes in the disease. Among its many pathologic hallmarks, glioblastoma has long been noted to have

significant vascular proliferation with the increased expression of pro-angiogenic factors.<sup>16</sup> Vascular endothelial growth factor (VEGF) is an important angiogenic factor that has been demonstrated to play an integral role in tumor angiogenesis.<sup>17</sup> Several anti-angiogenic agents targeting VEGF pathways have been successful in clinical trial for various human cancers.<sup>18,19</sup> In glioblastoma, VEGF has been demonstrated to be highly expressed, particularly in regions of tumor that are proximal to necrotic areas.<sup>20–23</sup> In fact, molecular studies have indicated that VEGF expression correlates well with both glioma grade and prognosis with higher VEGF expression associated with poorer prognosis.<sup>24</sup> Given the known role of VEGF in glioblastoma, studies in the late 1990s utilizing xenograft models of glioblastoma suggested that antibodies to VEGF could inhibit the growth and progression of the tumor.<sup>25</sup>

Bevacizumab (Avastin, Genentech) is a humanized monoclonal antibody that has its action against VEGF-A. As the first anti-angiogenesis agent approved for use in cancer, bevacizumab was initially demonstrated to be efficacious in metastatic colon cancer in a large phase III trial.<sup>26</sup> Subsequently, bevacizumab has been successfully used in metastatic non-small cell lung cancer and renal cancer.<sup>27,28</sup> With these successes and promising animal-studies work, bevacizumab was tested in patients with recurrent glioblastoma. A single-institution phase II study would demonstrate remarkable results with the use of bevacizumab and irinotecan, with a RR of 57% and a progression-free survival at 6 months (PFS-6) of 46% without excessive toxicity.<sup>29</sup> The promising results would be reproduced in the BRAIN trial, a multi-institutional, randomized, non-comparative phase II trial that examined the use of bevacizumab or the combination of bevacizumab with irinotecan in recurrent glioblastoma. In the BRAIN trial, published in 2009, patients exhibited a RR of 33% with a PFS-6 of over 40%.<sup>5</sup> These figures were a significant improvement over historical controls with systemic chemotherapies, which were estimated to be a RR of 5% and a PFS-6 of 15%.



Based upon these dramatic results, bevacizumab received accelerated approval by the Food and Drug Administration for the treatment of recurrent glioblastoma in 2009.<sup>30</sup> Despite the hope provided by bevacizumab, several limitations of its use have been noted over recent years. First and foremost, over half of recurrent glioblastoma patients fail to respond to bevacizumab, which underlines the continued need for better therapies for this disease.<sup>31</sup> Furthermore, response to bevacizumab is often short-lived as patients inevitably develop resistance to therapy.

The true benefit of bevacizumab remains unclear, especially because its ability to prolong overall survival (OS) is controversial. Compared to historical controls, it initially appeared that patients were receiving an OS benefit from bevacizumab.<sup>5</sup> A retrospective study would, however, subsequently suggest that anti-angiogenic agents do not prolong OS despite the dramatic effects on RR, PFS and PFS-6.<sup>8</sup> In fact, two large, phase III studies would support the notion that bevacizumab does not provide a survival benefit in glioblastoma, as both studies demonstrated known benefits in progression-free survival (PFS) without a subsequent benefit in survival.<sup>32,33</sup> The disparity between common clinical trial endpoints of RR, PFS and PFS-6 with OS raised awareness of the challenges in the neuro-imaging of glioblastoma, particularly in the setting of bevacizumab and other new anti-angiogenic agents.<sup>34,35</sup>

### *Imaging of Glioblastomas*

Prior to the advent of magnetic resonance imaging (MRI), computed tomography was the standard technique for the imaging of brain tumors. As early as 1981, MRI was noted to provide superior imaging of the brain parenchyma relative to computed tomography.<sup>36</sup> Technology would rapidly develop as the introduction of both gadolinium-based contrast agents and high-field scanners in the early 1980s dramatically improved the ability to visualize the brain.<sup>37,38</sup>

With continued improvement of MRI technology, anatomic MRI represents the principle means of non-invasively assessing glioblastoma throughout its disease course.<sup>39</sup>

Conventional MRI protocols for brain tumors has traditionally included T1-weighted, post-gadolinium T1-weighted, and T2-weighted imaging. Fluid-attenuated inversion recovery (FLAIR) sequences represent strongly T2-weighted images with the suppression of signal in the cerebrospinal fluid, and this has become a part of standard imaging protocols since the late 1990s.<sup>39</sup> Glioblastoma is classically characterized to be an intraparenchymal, heterogeneously contrast-enhancing, bulky mass with areas of central necrosis.<sup>40</sup> Glioblastoma is known to have abnormal vasculature with a blood-brain barrier that is leaky and permissive of contrast extravasation, which results in its characteristic contrast enhancing appearance on post-gadolinium images.<sup>41</sup>

The increased use of bevacizumab and other anti-VEGF agents have complicated the imaging of glioblastoma.<sup>34</sup> Bevacizumab causes a rapid decrease in the enhancement seen on MRI images of tumor, which is partly responsible for the high RR seen in clinical trials. Thus, the therapy actually alters the appearance of the tumor on MRI, but this does not necessarily represent anti-tumor activity of the drug.<sup>42</sup> This phenomenon has been termed pseudoresponse.<sup>43</sup> The issue of pseudoresponse raises the concern that conventional MRI imaging of contrast-enhancing tumor is an inadequate surrogate for actual tumor assessment.

Furthermore, animal studies have revealed that tumors being treated with anti-VEGF agents may progress by overcoming the inhibition of angiogenesis by assuming a more infiltrative phenotype and siphoning off existing brain vessels.<sup>44</sup> Since this type of progression cannot be seen with post-contrast T1-weighted imaging, it has been termed non-enhancing progression. Non-enhancing progression can be visualized on T2-weighted and FLAIR images as increasing areas of hyperintensity.<sup>45</sup> The use of bevacizumab has been associated with an

increasing incidence of nonenhancing progression that is apparent on T2/FLAIR imaging.<sup>45</sup> The lack of contrast enhancement has been suggested to represent the tumor's evolution into a more diffuse, infiltrating phenotype that can be detected on T2/FLAIR without changes on T1-weighted imaging.

In the setting of bevacizumab, pseudoresponse and non-enhancing progression represent major considerations when interpreting MRI imaging of glioblastoma. The prediction and assessment of response to bevacizumab are an important challenge that have been difficult to address with traditional response assessment criteria.<sup>46</sup>

## Study 1: Validation of the RANO Criteria

Given the emerging challenges in response assessments with neuro-imaging, clinical endpoints in glioblastoma have become an area of controversy in neuro-oncology.<sup>47</sup> Although overall survival (OS) is generally considered to be the “gold-standard endpoint”, the evaluation of therapy in phase II and III trials have historically also relied upon radiographic RR and PFS.<sup>35</sup> In clinical trials of recurrent glioblastoma, RR and PFS are particularly important because these endpoints are not confounded by salvage therapies and other variables that may affect OS.<sup>48,49</sup> Several studies utilizing clinical trial data have indicated that RR and PFS correlate with OS,<sup>7,50,51</sup> supporting their use as a reliable, efficient clinical endpoint in clinical trials.

Since 1990, the main response criteria used in clinical trials in neuro-oncology has been the Macdonald Criteria, which uses two-dimensional contrast enhancing area to assess tumor size, in addition to corticosteroid use and clinical status.<sup>52</sup> It involves assessment of the contrast enhancing lesion on post-contrast T1-weighted imaging by measuring the longest axis multiplied by the longest perpendicular measurement in any plane. Response is defined as  $\geq 50\%$  decrease in the bidimensional product. Progression is defined by  $\geq 25\%$  increase in the bidimensional product relative to the best response scan. Although it has been tremendously valuable for clinical trials, the Macdonald Criteria has a number of limitations in the setting of recurrent disease that have become increasingly problematic, especially due to phenomenon of pseudoresponse and nonenhancing tumor progression.<sup>48,50</sup>

To address several limitations of the Macdonald criteria, the Response Assessment in Neuro-Oncology (RANO) Working Group proposed newly updated response criteria for high-grade gliomas in 2010.<sup>53</sup> They were designed to determine better criteria of response assessment that could enhance the interpretation of clinical trials in the setting of novel therapeutics such as

angiogenesis inhibitors. The RANO criteria still uses two-dimensional contrast enhancing tumor as the basis of response, but it also accounts for T2/FLAIR nonenhancing tumor and provides guidelines on measurability, multifocal lesions, and pseudoprogression. The quantitative parameters for response and progression remain the same for contrast enhancing tumor. Of note, the RANO criteria did not specify quantitative thresholds for determining nonenhancing progression on T2/FLAIR imaging because of the difficulty in determining this by using standard two-dimensional measurements. Principles underlying the RANO criteria have been adopted into clinical trials involving high-grade gliomas, including the phase III AVAglio study, which was devised prior to publication of the RANO update.<sup>46</sup> Although a prior retrospective analysis comparing RANO, Macdonald and Response Evaluation Criteria in Solid Tumors (RECIST) indicated concordance among all of the one-dimensional and two-dimensional response assessment criteria among recurrent glioblastoma patients,<sup>54</sup> the RANO criteria has not yet been validated with outcomes data from a prospective trial.

### ***Study 1 Objective and Hypothesis***

In order to validate the RANO criteria as part of a multi-institutional effort, we analyzed the radiographic data of patients with recurrent glioblastoma being treated with bevacizumab or bevacizumab with irinotecan from the randomized phase II BRAIN trial<sup>5</sup> as a means to perform a comparative analysis of the Macdonald Criteria and the RANO Criteria with respect to RR and PFS. It was hypothesized that the RANO criteria would allow for a shorter PFS by identifying a subset of patients exhibiting nonenhancing progression. Further, we hypothesized that the surrogate endpoints of RR and PFS, as defined by the RANO criteria, would correlate with subsequent survival. Given the qualitative nature of determining nonenhancing progression per

the RANO criteria, inter-observer variability was investigated among readers from different institutions and different specialty training (i.e. neuroradiologists and neuro-oncologists).

## **Materials and Methods**

### *Patients*

All patients were from the phase II BRAIN trial,<sup>5</sup> which was performed to assess the effectiveness of bevacizumab with or without irinotecan in patients with recurrent glioblastoma. For this trial, 167 patients from multiple participating centers who had histologically confirmed glioblastoma at first or second relapse were enrolled. Disease progression that led to enrollment in the study was identified on MRI  $\leq 14$  days before the baseline treatment. These patients had failed the initial standard care plan including concurrent radiotherapy and temozolomide, and they were only enrolled if they were at least 8 weeks from the completion of radiation therapy. Bevacizumab was given at a dose of 10 mg/kg. The dose of irinotecan varied based on different clinical scenarios. All patients were treated for 104 weeks or until disease progression or discontinuation. Other inclusion criteria included Karnofsky performance status (KPS)  $\geq 70$  %; life expectancy  $\geq 12$  weeks; and adequate hematologic, hepatic, and renal function. Patients receiving corticosteroids were required to be on a stable or decreasing dose for at least 5 days before the baseline MRI scan. Of the 167 patients from the BRAIN study, 163 patients were used in the current analysis of PFS, based on the availability of both clinical data and pre-treatment T1 post-contrast images and T2-weighted imaging of sufficient quality.

All the images from the patients from the BRAIN study, including baseline MRI and follow-up MRIs performed every 6 weeks until progression or until the pre-determined clinical trial lock-date for imaging were obtained. Imaging and clinical data, including corticosteroid

dosing, were acquired from Genentech. Data acquisition was performed in compliance with all applicable Health Insurance Portability and Accountability Act regulations (HIPAA).

### *Radiological Response and Progression Assessment*

T1-weighted non-contrast and contrast-enhanced images and T2/FLAIR images were displayed on a picture archiving and communication system (PACS) workstation. For each patient, scans were read serially by a reader who was blinded to the number of scans available for each patient. The reader evaluated enhancing and nonenhancing tumor in order to determine response status and progression dates by Macdonald and RANO criteria. Only quantitative measurements of contrast enhancing tumor or new lesions qualified for progression by Macdonald criteria. Quantitative changes in T1-weighted gadolinium-enhancing lesion, as well as qualitative changes in non-enhancing T2/FLAIR, were used to assess treatment response and disease progression by RANO criteria. The dates of progression were determined by a radiologist blinded to clinical information. Once there was assignment of progression by RANO or Macdonald criteria, the reader was not able to modify the assigned progression date. Two-dimensional measurements of contrast enhancing and nonenhancing tumor were recorded for every available scan.

PFS and OS were calculated with respect to the date of bevacizumab initiation. Corticosteroid dosing was evaluated, and the PFS analysis was appropriately adjusted to account for increases in steroid dosing between imaging.

### *Inter-observer variability*

All available scans of 163 patients were read and measured by a board certified neuro-radiologist. The scans of 30 randomly selected patients were read by a board-certified neuro-

radiologist at a separate participating institution. The scans of 85 randomly selected patients were also read by board-certified neuro-oncologists in order to further assess variability.

### *Statistical analysis*

Statistical analysis was completed by an outside institution in this collaborative multi-institutional project. Kaplan-Meier estimates were used to calculate PFS and OS. Patients who did not progress were censored appropriately for the last scan date available. Patients who did not die were censored according to the last date known alive per the clinical data provided by Genentech. Correlative statistics were completed between PFS determined by the RANO and Macdonald criteria. The difference in PFS between RANO and Macdonald criteria was analyzed using log-rank testing. Response and progression were correlated to OS using landmark analyses at 2, 4 and 6 months with a Cox proportional hazards model implemented with response and progression treated as a time-dependent variable, respectively. Concordance statistics (C-index) was used to evaluate the predictive effects of PFS on OS with each respective criteria.

Inter-observer variation was gauged with correlative statistics of the PFS and the absolute measurements of enhancing and nonenhancing lesions among readers.

## **Results**

### *Patient characteristics*

Of the 167 patients (115 males) of the BRAIN trial, a total of 163 had available imaging. Patient characteristics, previously published,<sup>5</sup> are summarized in Table 1. Of note, the mean age was 56. A majority of patients (56%) had a KPS of 70-80, and 41% had a KPS score of 90-100. While 81% of patients were at first recurrence, 19% were at their second recurrence at time of enrollment into the clinical trial.



### *Response: RANO vs. Macdonald*

For the response analysis, 156 of 167 patients had baseline and post-treatment imaging available for analysis. RR was 53.8% and 55.6% by Macdonald and RANO criteria, respectively.

### *Correlating RR with overall survival*

Response as determined by Macdonald and RANO criteria were correlated with OS via landmark analysis completed at 2, 4, and 6 months (Figure 1).

Landmark analysis using response as determined by Macdonald criteria indicated that response was predictive of OS at all time points after adjustment for appropriate clinical variables. At 2 months, responders had a HR 1.44 (95% CI: 1.01-2.04, p-value =0.0419) relative to non-responders. The C-index at 2 months was 0.547 (+/- 0.026). At 4 months, the HR was 2.41 (95% CI: 1.61-3.59, p-value <0.0001) with a C-index of 0.618 (+/- 0.026). At 6 months, the HR was 2.82 (95% CI: 1.72-4.63, p-value <0.0001) with a C-index of 0.612 (+/- 0.028).

Landmark analysis using response as determined by RANO criteria indicated that response was also predictive of OS at all time points after adjustment for appropriate clinical variables. At 2 months, responders had a HR 1.48 (95% CI: 1.04-2.10, p-value=0.0274) relative to non-responders. The C-index at 2 months was 0.547 (+/- 0.026). At 4 months, the HR was 2.12 (95% CI: 1.41-3.20, p-value =0.0003) with a C-index of 0.595 (+/- 0.026). At 6 months, the HR was 2.11 (95% CI: 1.17-3.81, p-value=0.0128) with a C-index of 0.564 (+/- 0.024).

### *Progression: RANO vs. Macdonald*

For the PFS analysis, 163 of 167 patients had an available treatment start date and were used for the analysis. Of 163, 86 patients had progressed per Macdonald criteria, and 111

patients had progressed per RANO criteria. There were 37 patients who had a different progression date determined by RANO criteria relative to Macdonald criteria. Patients who did not progress were appropriately censored at last date of imaging without progression. Using Macdonald criteria, the median PFS was 5.52 months. By RANO criteria, the median PFS was 4.21 months. Comparing the PFS resulting from the two criteria by log-rank test, the RANO criteria elicited a statistically significant decrease in PFS in comparison to the Macdonald criteria (Figure 2,  $p=0.0423$ ).

#### *Correlating progression with overall survival*

Progression as determined by Macdonald and RANO criteria were correlated with OS via landmark analysis completed at 2, 4, and 6 months (Figure 3).

Landmark analysis using progression as determined by Macdonald criteria indicated that progression was predictive of OS at all time points after adjustment for appropriate clinical variables. At 2 months, progressors had a HR 2.77 (95% CI: 1.56-4.92,  $p$ -value =0.0005) relative to non-progressors. The C-index at 2 months was 0.538 (+/- 0.013). At 4 months, the HR was 3.53 (95% CI: 2.37-5.25,  $p$ -value <0.0001) with a C-index of 0.641 (+/- 0.022). At 6 months, the HR was 3.67 (95% CI: 2.41-5.58,  $p$ -value <0.0001) with a C-index of 0.677 (+/- 0.027).

Landmark analysis using progression as determined by RANO criteria indicated that progression was also predictive of OS at all time points after adjustment for appropriate clinical variables. At 2 months, progressors had a HR 3.19 (95% CI: 2.01-5.07,  $p$ -value<0.0001) relative to non-progressors. The C-index at 2 months was 0.578 (+/- 0.016). At 4 months, the HR was 4.08 (95% CI: 2.75-6.07,  $p$ -value <0.0001) with a C-index of 0.667 (+/- 0.023). At 6 months, the HR was 2.81 (95% CI: 1.85-4.26,  $p$ -value <0.0001) with a C-index of 0.675 (+/- 0.029).

### *Inter-observer variability*

Inter-observer variability was assessed by comparing the primary reader vs. other readers (other institutions and/or other specialties). As indicated in Table 2, there was significant correlation between primary reader and other readers for PFS via Macdonald ( $r=0.689$ ) and RANO ( $r=0.691$ ) criteria. There was also significant correlation between primary reader and other readers regarding the change in absolute measurements for the contrast-enhancing lesion ( $r=0.623$ ) and the nonenhancing lesion ( $r=0.544$ ).

After combining reads by everyone ( $n=278$ ), there was significant correlation in PFS between Macdonald and RANO criteria ( $r=0.781$ ).

## **Discussion**

In this study, we compared the Macdonald and the RANO criteria using the radiological data from the phase II BRAIN trial of patients receiving bevacizumab or bevacizumab and irinotecan for recurrent glioblastoma. We furthermore examined the relationship of commonly used trial endpoints of RR and PFS, as determined by each criteria, with subsequent OS, which is the accepted gold-standard of clinical trial end points in glioblastoma. Although there was no significant difference in RR between the criteria, our results indicate that the use of RANO criteria was associated with a small difference in PFS that was statistically significant with a difference of 1.3 months in median PFS in comparison to the Macdonald criteria ( $p=0.0423$ , Figure 2). Furthermore, the endpoints of response and progression per RANO criteria, as well as Macdonald criteria, correlated with OS.

### *Response Rate*

There were no significant differences in RR using Macdonald and RANO criteria. There were not many cases of patients who would exhibit a response on T1-weighted with contrast imaging who did not respond on the T2/FLAIR. Hence the study reproduced prior research in demonstrating that recurrent glioblastoma patients with a response while being treated with bevacizumab or bevacizumab with irinotecan have a longer survival compared to nonresponders.<sup>50</sup> Although a prior analysis of North Central Cancer Treatment Group clinical trials among recurrent glioblastoma patients did not elicit a statistically significant relationship between RR and OS,<sup>87</sup> there is a growing literature in recurrent glioblastoma patients initiated on bevacizumab that early response portends a better prognosis.<sup>7,88</sup> Our study further supports the notion that RR per RANO criteria can provide an early signal of anti-tumor activity and possibly an indication of durable benefit with anti-angiogenic agents.

Of note, the RR determined by our independent reads are much greater than reported by the independent radiology facility (IRF; RadPharm, Inc.) in the published BRAIN trial.<sup>5</sup> These differences are likely attributable to several nuances of measurement delineated in the RANO criteria regarding assignment of response status. The RANO criteria provides a clear definition of measurable disease and its distinction from nonmeasurable disease, which was different from what was employed by the independent radiological reads in the BRAIN trial. The variability exhibited in our RR relative to IRF remains a reminder of the challenges and variability of imaging-based endpoints in the setting of anti-angiogenic therapies.

### *Progression-free survival*

To our knowledge, this is the first study to indicate a statistically significant difference in PFS by using the RANO criteria in comparison to the Macdonald criteria. A prior, single-institution study in recurrent glioblastoma patients on bevacizumab had indicated that the use of

RANO trended towards a shorter PFS, but this did not reach statistical significance.<sup>54</sup> There were several differences between the Perez-Larraya et al. study and the present study that should be noted. The present study had a significantly larger sample (163 vs. 78) drawn from institutions across the country with strict trial inclusion criteria, which may allow for the detection of the modest improvement in PFS. Furthermore, the present study employed qualitative judgment of T2/FLAIR progression, as described by the RANO group. In contrast, Perez-Larraya et al. used quantitative measures to determine T2/FLAIR nonenhancing progression, which may increase objectivity but cannot detect subtle changes that can indicate nonenhancing progression at an earlier time point than two-dimensional measures. This difference in methodology may also explain the disparity in incidence of nonenhancing progression; the Perez-Larraya study identified 33% of patients exhibiting nonenhancing progression, while our analysis revealed 22% nonenhancing progression, which is more consistent with prior studies of T2/FLAIR nonenhancing, infiltrative progression.<sup>85</sup> The clinical relevance of the decrease in PFS is unclear. More importantly, there appears to be a subset of patients who demonstrate nonenhancing progression who benefit from being identified as progressors based upon T2/FLAIR signal. The RANO criteria appear to provide a clear benefit by identifying these patients as progressors so that clinicians and clinical trials can better approximate treatment failure.

Progression determined by Macdonald and RANO were both predictive of overall survival. This finding would support the notion that the PFS determined by the RANO criteria represents a valid end-point even in the setting of anti-angiogenic therapies. The results are in agreement with prior studies that have demonstrated that progression determined by Macdonald and modified Macdonald criteria correlate with survival.<sup>49,50,86</sup> The benefit of using PFS as a clinical trial endpoint is its ability to evaluate the efficacy of a particular treatment without being confounded by salvage therapies.<sup>35</sup> As previously suggested by Han et al. in a meta-analysis of

91 trials,<sup>51</sup> our study demonstrates that PFS determined by the RANO criteria remains a valid clinical trial endpoint in bevacizumab-treated patients. As PFS offers an opportunity for earlier outcomes assessment and higher statistical power at the time of analysis, our study supports its continued use as a surrogate endpoint of OS in recurrent glioblastoma clinical trials.

By using concordance (C) statistics in the landmark analysis, we were able to better evaluate predictive effects of our progression co-variables on subsequent overall survival. The landmark analysis 2, 4, and 6 months using both criteria were significant at all time points for progression, with C-index ranging from 0.538 to 0.677. Interestingly, the C-index for progression trended towards increased values at later time points, and it was highest at 6 months (Macdonald 0.677, RANO 0.675). In other words, a patient's progression status at 6 months was possibly more predictive of OS than progression status at an earlier time point. Although not directly tested, this would also support the use of progression-free survival at 6 months (PFS-6), which has been previously demonstrated by Lamborn et al. as a valid end point in trials for recurrent malignant glioma.<sup>49</sup> Of note, the C-index demonstrates *modest* predictive value of progression time points with Macdonald and RANO criteria. While this speaks to the value of using the RANO criteria to determine progression, developing better means of determining progression should allow for endpoints that are even more predictive of OS (C-index  $\geq 0.7$ ).

### *Variability*

There was a significant correlation in PFS done by the primary reader and other readers using both the Macdonald ( $r=0.688$ ) and RANO ( $r=0.692$ ) criteria. This is primarily attributable to the fact that only a relatively small subset of patients in the recurrent setting exhibit nonenhancing T2/FLAIR progression and are therefore affected by the RANO criteria. The RANO criteria inherently has a greater degree of variability associated with it because of the

subjective nature of determining nonenhancing, infiltrative progression. Among correlations of absolute measurements among readers, the correlation in the change of the nonenhancing lesion was lower in comparison to the correlation of measurements of the enhancing lesion.

Quantifying infiltrative, nonenhancing tumor is challenging because there are several causes of increased T2/FLAIR abnormal signal including radiation effects, decreased steroid dosing, demyelination, ischemic injury, infection, seizures, and post-operative changes.<sup>53</sup> For this reason, the RANO Working Group did not provide an objective way to determine T2/FLAIR progression.<sup>53</sup>

Given the subjective nature of nonenhancing progression per RANO criteria, it is important to establish the predictive effects of PFS as determined by RANO on OS to exclude the possibility of excessive overestimation of progression. As illustrated in Figure 3, there does not appear to be excessive overestimation of T2/FLAIR, as there is still clear stratification of survival by landmark analysis of progressors vs. non-progressors at all time points.

### *Study 1 Limitations*

There are several limitations that must be noted for this study. The BRAIN trial was a non-comparative phase II study in which all participants were treated with bevacizumab. There was no control arm and our conclusions cannot be applied to patients who do not receive bevacizumab. Furthermore, our time-to-event endpoints must be interpreted with caution given the retrospective nature of our analysis. While our studies support the use of RR and PFS as determined by the RANO criteria in clinical trials, larger prospectively designed studies are warranted. This is especially relevant because two recent large phase III clinical trials for newly diagnosed glioblastomas failed to demonstrate a survival benefit with the use of bevacizumab.<sup>32,33</sup>

Despite attempts to simulate real-life clinical decision making, the study was limited by its post-hoc design. Readers were blinded to clinical data as well as the total number of scans available for each patient. Hence, readers would have to decide upon progression without the knowledge of whether more scans were available, and this was implemented to reduce bias. Nonetheless, it was an experimental setting that failed to truly replicate clinical practice. Reader fatigue may also have contributed since readers would serially go through many patients in a single sitting. Other limitations include the fact that imaging was only available up to progression or up to a pre-specified lock-date that was built into the trial design. Statistical analysis accounted for this by censoring appropriately based upon the available imaging

#### *Future Directions for Response Assessment*

As alluded to, the RANO criteria currently utilizes a qualitative approach to determining T2/FLAIR nonenhancing progression. There would be clear advantages to developing a reliable, quantitative means of identifying T2/FLAIR nonenhancing, infiltrative progression. The RANO criteria was developed with the intention of remaining open to modifications so that newer techniques can be incorporated in the future. Ongoing research has been evaluating the potential use of advanced imaging in formal response assessment with promising preliminary results. While conventional linear measurements have been attempted to quantify changes in T2/FLAIR nonenhancing tumor,<sup>85</sup> advanced MRI techniques may be more helpful moving forward.

One research area of great potential is the viability of volumetrics for assessing glioblastoma instead of traditional two-dimensional measurements.<sup>60,88</sup> Glioblastomas are notoriously difficult to measure due to their irregularity, which is only compounded by asymmetrical shape, necrosis, cystic areas, and treatment-related effects. Several studies have examined the use of volumetric approaches to response assessment in comparison to two-



dimensional measurements.<sup>89,90</sup> In a study of 104 patients with an enhancing glioma, Shah et al. demonstrated that one-dimensional, two-dimensional and volumetric measurements were comparable in determining progression.<sup>89</sup> These findings would be reproduced with the imaging from a large clinical trial dataset.<sup>90</sup> Of note, these studies were completed prior to the widespread adoption of anti-angiogenic agents, and they did not evaluate the use of volumetric analysis to quantify T2/FLAIR volume. At this point in time, volumetric approaches have not been sufficiently standardized nor validated in order to be seriously considered for incorporation into formal response assessment criteria. Nonetheless, they continue to be an important area of research that may play a role in response assessment in the future.

Advanced MRI techniques such as DWI-MRI, PWI-MRI, and MRS, along with PET imaging, will require further study prior to consideration to become a standard part of response assessment. As subsequent studies delve into the use of advanced imaging in bevacizumab-treated recurrent glioblastoma patients, further discussion is deferred.

Finally, measures of quality of life, neurocognitive function or steroid use have been discussed as possible clinical end points that may eventually be able to be incorporated into response assessment.<sup>53</sup> There are currently ongoing efforts to standardize neurologic assessment of high-grade glioma patients in order to provide a standardized tool to assess clinical status. If validated,<sup>91</sup> these are planned to be incorporated into the RANO criteria.

### *Study 1 Conclusion*

The use of RANO criteria, in comparison to the Macdonald criteria, yields a shorter PFS because of its earlier identification of T2/FLAIR non-enhancing progressors. Response and progression determined by the RANO criteria were predictive of subsequent OS. Based upon these findings, it appears that RR and PFS determined by RANO criteria are reasonable end

points for clinical trials of therapy in glioblastoma. The future development of quantitative approaches to evaluating T2/FLAIR nonenhancing progression will be helpful in reducing variability inherent in the RANO criteria.

# **Prognostic and Predictive Imaging Biomarkers in Recurrent Glioblastoma**

Glioblastoma patient survival varies widely, and it is influenced strongly by patient age, performance status, completeness of resection and other factors.<sup>55–57</sup> While modern molecular diagnostics are exposing additional layers of genetic, epigenetic and other molecular variability underlying this striking phenotypic heterogeneity, prediction of treatment response and survival in individual patients remains a critical need. This need is particularly relevant in patients with recurrent glioblastoma where there appears to be a subset of patients who have a durable benefit from bevacizumab.

Hence, in addition to assessing response and progression, evaluating predictive and prognostic biomarkers that can identify patients who are likely to benefit from bevacizumab or other anti-angiogenic agents prior to or early in treatment would be tremendously helpful in order to better tailor therapy for patients and improve outcomes. With further analysis of conventional MRI and the development of MRI pulse sequences that are sensitive to physiology, new imaging-based biomarkers have been proposed for this purpose.

In order to overcome limitations of conventional MRI, a wide range of advanced MR and metabolic imaging techniques have been introduced that examine prognostic and predictive imaging biomarkers including MR spectroscopy (MRS), perfusion-weighted MRI (PWI-MR), and metabolic positron emission tomography (PET) imaging.<sup>58</sup> While increasingly utilized in large academic centers, the most common imaging sequences that are widely implemented remain conventional MRI and diffusion-weighted MRI (DW-MRI).<sup>39</sup> Our studies focus upon the use of parameters derived from conventional MRI and DW-MRI, but several other advanced

imaging techniques are being increasingly explored in bevacizumab-treated recurrent glioblastoma patients. It is worthwhile to review these methods.

Perfusion imaging, PWI-MR, allows for routine non-invasive evaluation of tumor vascularity and angiogenesis, and it primarily uses normalized cerebral blood volume (nCBV) as a marker for the presence of large dysplastic neovessels induced by VEGF and other vascular growth factors.<sup>105,106</sup> The relative amount of increased perfusion in gliomas has been correlated with tumor grade, as well as a prognostic marker for PFS and OS in newly diagnosed glioblastomas.<sup>66,107–109</sup> Sorensen et al. has described a “vascular normalization index” as a potential biomarker derived from PWI-MR that can predict response to a single dose of anti-angiogenic therapy (cediranib) and predict for PFS and OS in recurrent glioblastoma patients.<sup>110</sup> With increasing evidence in pilot studies that perfusion MR can be used as a predictor of bevacizumab response in recurrent glioblastoma,<sup>111–113</sup> a recent study by Schmainda et al. supported previous findings by demonstrating that PWI-MR can be used to predict response to bevacizumab while stratifying for PFS and OS in a single-institutional retrospective study of 36 patients.<sup>101</sup>

MRI spectroscopy (MRS) provides valuable information about the tissue composition of tumor. Advanced methods have allowed for quantification of tumor metabolism markers including glucose, choline, creatine and N-acetyl-aspartate. While several studies have indicated great potential in utilizing MRS in the setting of anti-angiogenic agents, evidence for a role in better predicting and assessing response to bevacizumab therapy is sparser relative to other advanced MR techniques. In a small sample of 13 patients in a recurrent glioblastoma clinical trial, Ratai et al. demonstrated that changes in the ratio of N-acetylaspartate to creatine as early as 2 weeks after bevacizumab initiation was associated with antitumor effect.<sup>102</sup> Further research will be necessary to better establish the role of MRS in this treatment setting.

PET imaging has been heavily studied in recurrent glioblastoma given its ability to provide information about metabolism and cell proliferation. The most commonly used radiotracer, 18-F-FDG can provide a surrogate for glucose uptake by cells, but high background uptake by the brain makes PET using this tracer difficult to interpret for glioblastoma. While a variety of tracers have been utilized, amino-acid based PET has been shown to be particularly useful as an imaging biomarker for predicting treatment response in brain tumors.<sup>58</sup> In a prospective pilot study of 21 recurrent glioblastoma patients treated with bevacizumab and irinotecan, Chen et al. indicated that PET imaging using 18-fluorothymidine (18-FLT-PET) as a tracer at 2 weeks and 6 weeks after bevacizumab initiation generated predictors of OS that was superior to responses elicited on conventional MRI.<sup>100</sup> Subsequent preclinical and clinical studies support the findings,<sup>114,115</sup> but larger studies are needed to better investigate the utility of PET imaging as an imaging biomarker in this setting. It must also be noted that PET imaging is particularly challenging because it is more costly and less widely available compared to MRI. In the absence of definitive evidence that PET imaging provides biomarkers with clear advantages over MRI, it will be difficult to incorporate this into routine clinical use for recurrent glioblastoma patients in the near future.

## **Study 2: Volumetric Assessment and Stratification of Patient**

### **Survival**

Tumor volume assessed by a volumetric analysis on conventional MRI sequences represents a promising biomarker that has not been thoroughly explored in the literature. While prior studies suggest that post-operative tumor volume and extent of resection have prognostic significance in *newly diagnosed* glioblastomas,<sup>59,60</sup> the importance of tumor volume in patients with recurrent tumor receiving anti-angiogenic therapy has not been extensively studied. In patients with recurrent tumor receiving bevacizumab, results from a phase II clinical trial have shown that 4-week post-treatment response based on two-dimensional measurement correlates with PFS.<sup>7</sup> However, no studies to our knowledge have demonstrated the utility of using early post-treatment tumor volume and response for predicting PFS and OS.

The use of three-dimensional volumes for recurrent glioblastomas is appealing because the tumors are challenging to measure with traditional two-dimensional methods. Glioblastomas classically exhibit irregular geometry, multifocality, and cystic or necrotic regions. Volumetric methods have the advantage of more reproducibly and precisely measuring the size of tumor,<sup>60-62</sup> which has led to an increasing interest of its utility in this context. For example, volumetric measurement of both the enhancing and non-enhancing tumor have been investigated as imaging markers for treatment response,<sup>45</sup> PFS, and OS.<sup>60,63</sup>

### ***Study 2 Objective and Hypothesis***

In order to examine volumetric analysis as a means of identifying patients likely to have a durable response to bevacizumab, we analyzed the radiographic data of patients with recurrent glioblastoma being treated with bevacizumab in an institutional database. We assessed whether

measurement of enhancing and nonenhancing tumor volume based on MRI at baseline and 3-6 week post-treatment initiation are useful predictors for PFS and OS. It was hypothesized that tumor volume and percentage change of tumor volume would allow for stratification of patients for PFS and OS.

## **Materials and Methods**

### *Patients*

The institutional review board approved this retrospective study with a waiver for informed consent. Using a pharmacy database, we retrospectively identified 252 patients with pathologically confirmed glioblastoma (WHO IV) who received bevacizumab at our institution between December 2005 and July 2012 with recurrence based on clinical and imaging data. Recurrence was defined by new or increased size of enhancing tumor (>25% bidimensional products) based on MRI prior to bevacizumab initiation. In order to be eligible, patients must have received bevacizumab with or without concurrent chemotherapy after failing no more than 3 prior treatment regimens, including standard radiation and temozolomide therapy. They must have also undergone pre-treatment MRI within 2 weeks preceding bevacizumab initiation and a follow-up MRI 3 to 6 weeks after bevacizumab initiation. Interpretable FLAIR, post-gadolinium T1-weighted, and DWI-MR sequences performed on either 1.5 Tesla or 3 Tesla MRI systems were also required at both imaging time points. Using these screening criteria, a total of 91 patients were selected.

### *Disease Progression Assessment*

RANO criteria, including changes in T1-weighted gadolinium-enhancing lesions, as well as non-enhancing T2/FLAIR areas of abnormality, were used to assess disease progression.<sup>53</sup> PFS and OS were calculated with respect to the date of bevacizumab therapy initiation.

#### *Enhancing and Nonenhancing Lesion Volume Segmentation*

Whole brain post-contrast T1 weighted and T2/FLAIR images from MRI obtained at baseline and after initiating bevacizumab were used for tumor volume segmentation. All tumor segmentations were done using 3D Slicer Software (version 4.1, Boston, MA)<sup>81,82</sup> by an investigator who was blinded to clinical outcomes. User-driven manual active contour segmentation was utilized to acquire volume quantification for the regions of interest in the enhancing tumor target(s) (T1+C) as well as T2/FLAIR abnormalities. Manual editing of tumor contour was also performed to exclude non-tumor regions such as areas of intrinsic T1 shortening, necrosis, or surgical cavity. Image volume was calculated by adding the number of pixels within volume contour and multiplying by pixel area and slice spacing. For multifocal tumors, the volume of separate lesions was summed together. Hence for each patient, a baseline and post-treatment enhancing and 'T2/FLAIR' volume was calculated. The percentage change in volume was calculated for both the enhancing and T2/FLAIR volume measurements for each patient. Also, the relative non-enhancing tumor ratio (rNTR), originally described by Norden et al. was calculated for both the baseline and post-treatment scans, respectively.<sup>8</sup>

#### *Statistical analysis*

The primary outcome measures were PFS and OS. The Kaplan-Meier method was used to provide median point estimates and time specific rates. The Cox proportional hazards model was used in uni-variable and multi-variable settings to identify volumetric markers significantly



associated with PFS and OS. Following analysis of clinical and volume parameters in this fashion, the sample was dichotomized by the median of the sample for each volume parameter. Appropriate subanalyses were planned to account for significant clinical variables found in the initial analyses. All of the statistical analyses were performed using STATA, version 12.0 (College Station, TX).

## **Results**

### *Patient clinical characteristics*

A total of 91 patients were selected using these screening criteria (51 males). Among 11 out of 91 patients who initiated bevacizumab within 12 weeks of the completion of concurrent chemoradiotherapy, tumor recurrence was supported by pathological confirmation on repeat resection (1 patient), detection of new satellite lesion (1 patient), PET imaging (2 patients), or marked clinical deterioration (7 patients). There were 12 patients with multifocal disease. Forty-seven patients were initiated on bevacizumab monotherapy, while 44 initiated bevacizumab with concurrent therapy. The concurrent therapies utilized in conjunction with bevacizumab included irinotecan (29 patients), temozolomide (6 patients), carboplatin (2 patients), carmustine or lomustine (4 patients), plerixafor (1 patient) and panobinostat (1 patient).

At the time of initial diagnosis, all patients were treated with temozolomide and radiotherapy following maximal tumor resection. The mean age of patients was 56.3 years old (range 23-88). Of 91 total patients, 47 patients (51%) were initiated on bevacizumab on first recurrence, 29 (32%) on second recurrence, 11 (12%) on third recurrence and 4 (4%) on fourth recurrence. The baseline MRI was obtained a mean of 4.0 days (+/- 4.0) before bevacizumab initiation, and the follow-up MRI was obtained a mean 30.0 days (+/- 6.0) after bevacizumab initiation. In terms of steroid usage, 51 patients were receiving dexamethasone (dose range

0.25mg-24mg, median 4mg) at the time of the baseline imaging. Forty-nine patients were receiving dexamethasone at time of immediate post-treatment scan (dose range 0.5mg-30mg, median 4mg). There were 16 patients who had an increased steroid dose at time of immediate post-treatment scan, while there were 49 and 26 patients with stable and decreased dexamethasone dosing, respectively. At the time of analysis, 70 patients died, and 85 patients had progressed per RANO criteria.<sup>53</sup>

The patient characteristics and baseline clinical variables with respect to PFS and OS are summarized in Table 3. Among clinical variables, number of recurrences and change in steroid dose from baseline to post-treatment scan were associated with PFS and OS.

*Univariable and multi-variable analyses of imaging parameters as continuous variables adjusted for clinical parameters.*

Age, number of recurrences, change in steroid dose, treatment regimen (bevacizumab monotherapy vs. concurrent chemotherapy) were included in a Cox proportional hazards model for evaluation with each imaging-based volumetric parameter (Table 4). Among radiologic variables, baseline enhancing volume, post-treatment enhancing volume, percent change, and post-treatment FLAIR volume were associated with OS. Post-treatment enhancing volume and percent change of enhancing volume were also associated with PFS. The rNTR was not predictive of OS or PFS. In multi-variable analysis investigating baseline and post-treatment volume parameters, post-treatment enhancing volume remained significantly associated with OS and PFS while the baseline parameters did not (Table 5).

Dichotomization of volumetric parameters using with sample median

*Enhancing volume parameters versus OS and PFS*

For each volume parameter, we dichotomized values by the sample median and then calculated Kaplan-Meier estimates of overall survival. The results for dichotomized enhancing volume parameters with hazard ratios (HR) from Cox proportional hazards model adjusted for age, use of steroids (yes or no), number of recurrences, and treatment regimen (bevacizumab monotherapy vs. bevacizumab with concurrent chemotherapies) are summarized in Table 6. When dichotomizing our sample by median baseline enhancing volume ( $19.46 \text{ cm}^3$ ), the baseline enhancing volume was predictive of OS ( $p=0.005$ ) but not PFS ( $p=0.077$ , Figure 4A). When dichotomizing our sample by median post-treatment enhancing volume ( $7.8 \text{ cm}^3$ ), the post-treatment enhancing volume was predictive of OS ( $p<0.001$ ), as well as PFS ( $p=0.018$ , Figure 4B). When dichotomizing our sample using median percentage change in enhancing volume (52%), the percentage change was also predictive of OS and PFS ( $p=0.009$  and  $p=0.001$ , respectively, Figure 4C).

Since a 64% reduction in enhancing volume numerically extrapolates to a 50% reduction in two-dimensional bi-dimensional product and therefore is equivalent as a partial or complete response per RANO criteria,<sup>53</sup> we also examined this threshold in a similar analysis. Accordingly, patients with a greater than 64% reduction in enhancing volume also associated with longer OS and PFS ( $p=0.004$  and  $p=0.024$ ).

#### *T2/FLAIR volume parameters versus OS and PFS*

Baseline, post-treatment, and percentage change of T2/FLAIR volume was not predictive of OS or PFS when dichotomizing our sample by median value for each parameter ( $p>0.05$ ).

#### *Combined stratifications using percentage volume change and residual enhancing tumor volume*

To examine the value of using both percentage volume change and residual enhancing volume, we first dichotomized the total patients group using 52% percentage change in enhancing volume, followed by dichotomization of each subgroup based on median residual enhancing volume of 7.8 cm<sup>3</sup>. Kaplan-Meier estimates of overall survival were calculated for each of these groups (Figure 5). Based upon the Cox proportional hazards model, residual enhancing volume was still a significant predictor of OS within the subgroups of both responders and nonresponders, although not a predictor of PFS. Of these four groups, patients who were responders per enhancing volume reduction, defined as a  $\geq 52\%$  response with a post-treatment enhancing volume of  $< 7.8\text{cm}^3$  had the highest median overall survival, 70.3 weeks. The lowest median overall survival was of patients who were non-responders with a post-treatment volume of  $> 7.8\text{cm}^3$ , as the median overall survival for this group was 21.1 weeks.

#### *Subgroup analysis: Recurrences*

Since the number of recurrences was a significant predictor of overall survival in uni- and multi-variable analyses, we examined volume parameters in subsets of patients at 1st and 2nd recurrence, respectively.

For patients at first recurrence (n=47), neither baseline enhancing volume nor percentage change were able to stratify the sample for survival using the previously listed median values for each parameter (p=0.294 and p=0.068, respectively). Dichotomization by post-treatment enhancing volume however, remained statistically significant (HR 1.98, p=0.044).

For patients at second recurrence (n=29), neither baseline enhancing volume nor percentage change were able to stratify the sample for survival (p=0.168 and p=0.289, respectively). Dichotomization by post-treatment enhancing volume however, again remained statistically significant in patients (HR=2.83, p=0.035).

### *Subgroup analysis: Bevacizumab monotherapy vs. bevacizumab with concurrent therapies*

In order to account for effects of heterogeneous concurrent therapies with bevacizumab given to some patients, we examined volume parameters among the subset of patients who received only bevacizumab monotherapy and those who received concurrent chemotherapy with Kaplan-Meier estimates. For patients with bevacizumab monotherapy (n=47), baseline enhancing volume was not able to stratify the sample for survival (p=0.118). Patients with greater than 52% change or greater than 7.8cm<sup>3</sup> at post-treatment were associated with longer overall survival (p=0.024 and p<0.001, respectively) in this subset.

For patients with concurrent therapy in combination with bevacizumab (n=45), only baseline enhancing volume was able to stratify the sample for survival (HR 2.14, p=0.033). Neither percentage change of enhancing nor post-treatment enhancing volume parameters, dichotomized by median, were able to stratify by survival (p=0.177 and p=0.059, respectively).

## **Discussion**

We assessed whether MRI-derived volume parameters prior to and early in treatment are useful predictors of outcome in patients with recurrent glioblastoma being initiated on bevacizumab. Our results show that residual enhancing tumor volume and percentage change of enhancing tumor volume from baseline are both associated with PFS and OS. Specifically, patients with residual enhancing tumor volume less than 7.8cm<sup>3</sup> have a longer median PFS (20.9 weeks vs. 12.0 weeks) and OS (64.1 weeks vs. 27.7 weeks). Similarly, patients with >52% percentage reduction of enhancing volume on post-treatment scan compared to baseline also have a longer median PFS (20.9 weeks vs. 11.9 weeks) & OS (52.3 weeks vs. 31.0 weeks). Our

results support the potential use of quantitative volumetric analysis early during bevacizumab to identify patients who may benefit more durably.

Radiographic response has been previously shown to be a predictor of OS at 9, 18 and 26 weeks using data from the phase II BRAIN Trial, although radiographic response was not a predictor of PFS in this study.<sup>5,50</sup> Another phase II trial of bevacizumab among heavily pretreated patients with recurrent glioblastoma indicated that early response at 4 weeks based on the Levin response assessment criteria is associated with a prolonged PFS. In this study early response based on objective Macdonald criteria did not demonstrate the same association.<sup>7,92</sup> Our results indicate that the percentage of change at an early point in treatment may serve as a predictor for both PFS and OS. If one is to extrapolate the percentage change threshold from 2D to 3D, a 50% change in bidimensional products approximates a 64% change in volume. Consistent with two-dimensional studies, we demonstrated that percentage change in volume ( $<$  or  $\geq 64\%$ ) can also stratify patients with respect to PFS and OS. The robustness of our stratification may result from the advantages that come with a volumetric approach. By avoiding areas of necrosis, cysts and surgical cavity, volumetric segmentation may potentially estimate tumor size more accurately than linear methods.<sup>27-29</sup>

The same predictive significance of percentage change of enhancing tumor was not observed in a previous study using a volumetric approach.<sup>63</sup> As a distinguishing feature, our study included patients with a post-treatment MRI scan performed between 3 to 6 weeks; in contrast, the post-treatment scan was obtained after 6 to 8 weeks in the Ellingson et al. study. It is possible that the anti-permeability effect of bevacizumab may have a greater impact on the measurement of enhancing tumor volume when it is measured at a later time point in therapy. If so, the percentage change in volume of enhancing lesion over a longer duration may less accurately reflect underlying tumor activity. Interestingly, in a different study by the same group which was

subsequently completed, Ellingson et al. demonstrated results concordant with our own.<sup>93</sup>

Residual enhancing volume appears to be associated with PFS and OS, but it was not clear if this association was indirectly related to the percentage change in enhancing volume since these variables are interrelated. To investigate potential interactions between post-treatment and percentage volume change, we used residual enhancing volume ( $< \text{or } \geq 8 \text{ cm}^3$ ) to further stratify patients who had an early response (defined as  $> 52\%$  reduction of enhancing volume) and patients without an early response. This created four subgroups from the original patient cohort (Figure 5). There is a significant difference in median OS between the subgroups in pairwise comparisons. The difference in PFS was not significant between the subgroups, possibly due to smaller sample size following combined stratifications. These findings suggest that even if there is interaction between residual enhancing tumor volume and percentage volume change, the combined use of both parameters appears to further stratify patients with respect to OS. Specifically, patients with a response ( $> 52\%$  reduction in enhancing volume from baseline) and small residual enhancing tumor volume ( $< 7.8 \text{ cm}^3$ ) had the longest median survival (70 weeks). On the other hand, nonresponding patients ( $< 52\%$  change in enhancing volume) and large post-treatment tumors (residual enhancing tumor volume  $> 7.8 \text{ cm}^3$ ) had the shortest median survival (21 weeks). Patients who were responders and yet still had large residual enhancing tumor and patients who were non-responders but small residual enhancing tumor had an intermediate median survival (28 weeks and 45 weeks, respectively).

Given the current controversy about appropriate timing of bevacizumab treatment in patients with recurrent glioblastoma, it is important to assess volumetric parameters as a function of the number of recurrences to better clarify their contribution to overall outcome. As the number of recurrences was significantly associated with PFS and OS in univariable and multivariable settings, we conducted an analysis stratified by this variable. In the patient

subgroup with one recurrence (n=47), residual enhancing tumor volume and percentage change in enhancing volume remained predictive of OS. In patients with exactly two recurrences (n=29), only residual enhancing volume was predictive of OS, while percentage change was not. These parameters were also mostly not significant for PFS, which may be attributable to the relatively small sample size in each subgroup.

The phenomenon of nonenhancing progression despite stable or improved enhancement following anti-angiogenic therapy, has led to the inclusion of consideration of the T2/FLAIR nonenhancing component of tumor in the RANO criteria as described in Study 1. For this reason, we also assessed T2/FLAIR tumor size using volumetric analysis in this study. While univariable analysis indicated that post-treatment T2/FLAIR volume correlated with PFS and OS, multivariable analysis indicated that T2/FLAIR is not significant following adjustment of clinical and other volumetric parameters. In this sample of heavily pre-treated patients, T2/FLAIR changes related to prior surgery, radiation and chemotherapy rather than underlying tumor activity may have contributed to measured T2/FLAIR volume as all abnormal signal was measured regardless of etiology.

Since the publication of our findings,<sup>88</sup> there have been several studies that have supported and enhanced our findings. Ellingson et al. have demonstrated that the use of subtraction maps of images that subtract intensity-normalized nonenhanced T1-weighted images from the contrast-enhanced T1-weighted images may allow for easier and more reliable volumetric segmentation of true enhancing tumor.<sup>93</sup> The study, utilizing the imaging from a multi-institutional clinical trial of bevacizumab-treated patients, confirms our findings that absolute residual volume and response can stratify for PFS and OS. Furthermore, while we employed manual contouring of tumors, this represents a time-consuming technique that is open to inter-observer variability. There is increased evidence that newer semi-automated approaches



are faster and more reproducible.<sup>94</sup> There has also been demonstration of improved ability to segment T2/FLAIR volumes specifically for nonenhancing tumor,<sup>95,96</sup> though the significance of this as a predictive biomarker is not clear.

### *Study 2 Limitations*

First, as a retrospective analysis, our study findings will require prospective evaluation. In this study, we cannot determine whether the residual and percentage change of enhancing volume are predictive of prognostic because all patients in our cohort received bevacizumab. To determine whether the volumetric imaging markers are specific to anti-angiogenic therapy, the same analysis should be investigated in recurrent glioblastoma patients treated without inhibitors of angiogenesis. In the absence of this comparison, the residual enhancing volume and percentage change of enhancing tumor volume cannot be definitively considered as predictors of bevacizumab therapy. Of note, while every patient in this study was initiated on a treatment regimen including bevacizumab, nearly half were also given concurrent chemotherapy.

The MRI sequence used to assess tumor volume, as dictated by institutional protocols, had an inter-slice thickness of 5 mm, potentially introducing partial volume averaging during the tumor segmentation. While high-resolution 3D T1-weighted MR images would be expected to allow for a more precise volume measurement, a prior study indicated that performing volumetric analysis on traditional 2D imaging, as done in this study, leads to comparable measurements.<sup>97</sup> Nonetheless, if quantitative volumetric analysis is to be implemented clinically to inform clinical management, tumor volume measurement based on 3D source imaging will be preferred. With advances in imaging acquisition speed, 3D T1- and T2-weighted whole brain image sequences are increasingly acquired in both trial and clinical settings and will be helpful in allowing for more accurate volume measurements.

### *Study 2 Conclusion*

We assessed baseline and early post-treatment volumetric parameters from T1-weighted and T2-weighted MRI imaging to evaluate their ability to predict PFS and OS in patients with recurrent glioblastoma being initiated on bevacizumab therapy. Volumetric percentage change and early post-treatment volume of enhancing tumor can stratify survival for patients with recurrent glioblastoma receiving bevacizumab therapy. T2/FLAIR abnormality volume was not able to stratify patients using this approach. Prospective validation of findings is warranted.

## **Study 3: ADC Histogram Analysis of Enhancing and Nonenhancing Tumor\***

The described study has been previously published in *Cancer*.

DW-MRI examines tissue by probing water mobility to indirectly assess cell density and tissue architecture. Given its relatively short acquisition time and high sensitivity to various brain pathology, this technique is commonly included as part of routine protocol for clinical imaging of brain tumors. The apparent diffusion coefficient (ADC) values derived from DW-MRI are thought to be primarily determined by cell density; lower ADC values correspond to higher cell density, while higher ADC values occur in areas of edema and necrosis. ADC values of low-grade gliomas are often greater than those of the normal brain tissue but not significantly different from the ADC values of peritumoral edema.<sup>64,65</sup> In high-grade gliomas, however, lower ADC values are observed as a result of greater cellularity.<sup>66</sup>

Due to the regional variability commonly observed in gliomas including areas of necrosis, infiltrative tumor, edema and post-radiation change, it is difficult to exclude sampling bias when analysis is performed without taking the entire tumor volume into account. Thus, several recent studies have characterized diffusion imaging using volumetric segmentation methods in gliomas.<sup>67–80</sup>

These studies can be divided into those performing histogram analysis of diffusion data within the volume-of-interest from pretreatment MRI,<sup>67,68,75,78,79</sup> and those using a voxel-subtraction functional diffusion map (fDM) technique tracking regional change between two different time points during the course of therapy.<sup>70,73,74,80</sup> Both techniques have demonstrated promising findings relating diffusion characteristics with treatment response and patient survival.

Functional diffusion maps (fDMs), described in gliomas as early as 2005, are created by examining voxel-wise changes in ADC value.<sup>76</sup> ADC maps from specific time points in therapy are co-registered so that values can be compared over different time points. Prospective studies in newly diagnosed patients with high grade gliomas (anaplastic astrocytoma or glioblastoma) utilizing fDMs have demonstrated that greater increases in diffusion were exhibited in patients with a longer OS.<sup>73</sup> This finding has been extended to patients with recurrent glioblastoma being initiated on bevacizumab,<sup>69</sup> indicating that fDMs can be utilized to stratify patients for OS. Advances such as using graded fDMs, where the absolute change in ADC is taken into account, and non-linear registration of images are techniques that have demonstrated even greater sensitivity and specificity for predicting OS.<sup>77</sup>

As a more recently devised approach for glioblastoma, histogram analysis of ADC values has been utilized to stratify patients being initiated on bevacizumab for PFS.<sup>67</sup> Specifically, curve fitting with a double Gaussian model has been demonstrated to be effective as a imaging biomarker by dividing ADC values within tumor into a low ADC and high ADC peaks. These findings would subsequently be reproduced in a validation study that used a clinical trial imaging dataset by demonstrating that the pre-treatment ADC could stratify patients for OS.<sup>78</sup> Of particular interest, it appears that the use of pre-treatment ADC as a predictive biomarker is only applicable for recurrent glioblastoma patients being initiated on bevacizumab. Patients receiving an alternative systemic chemotherapy without an anti-angiogenic agent for recurrent glioblastoma could not be stratified with the pre-treatment ADC histogram analysis.<sup>79</sup>

While most prior analyses of diffusion imaging of high-grade gliomas focus on the volume of enhancement, there is evidence that characterization of ADC within the entire volume of T2/FLAIR abnormality can stratify patients in terms of survival.<sup>70,71</sup> Furthermore, to our knowledge, characterization of ADC histograms within the nonenhancing T2/FLAIR

abnormality using multi-component curve-fitting has not been previously performed. It is unclear whether the analysis of the non-enhancing component of tumor can improve stratification of patient survival over analysis of enhancing tumor alone.

### ***Study 3 Objective and Hypothesis***

In order to examine the role ADC histogram analysis in the T2/FLAIR nonenhancing component of tumor as an imaging biomarker, we analyzed the radiographic data of patients with recurrent glioblastoma being treated with bevacizumab from an institutional database prior to therapy and early in treatment (3-6 weeks). Based upon our prior study employing volumetrics, we hoped to apply examine the role of ADC histogram analysis within enhancing and nonenhancing tumor volumes. We hypothesized that parameters derived from multi-component curve-fitting of ADC histograms within nonenhancing T2/FLAIR abnormality can be combined with those derived from enhancing tumor to improve stratification of PFS and OS.

## **Materials and Methods**

### ***Patients***

The same single institutional database of recurrent glioblastoma patients being initiated on bevacizumab used in Study was analyzed in this study. As previously described, the institutional review board approved this retrospective study with a waiver for informed consent. Using a pharmacy database, we retrospectively identified 252 patients with pathologically confirmed glioblastoma (WHO IV) who received bevacizumab at our institution between December 2005 and July 2012 with recurrence based on clinical and imaging data. Recurrence was defined by new or increased size of enhancing tumor (>25% bidimensional products) based on MRI prior to bevacizumab initiation. In order to be eligible, patients must have received

bevacizumab with or without concurrent chemotherapy after failing no more than 3 prior treatment regimens, including standard radiation and temozolomide therapy. They must have also undergone pre-treatment MRI within 2 weeks preceding bevacizumab initiation and a follow-up MRI 3 to 6 weeks after bevacizumab initiation. Interpretable FLAIR, post-gadolinium T1-weighted, and DWI-MR sequences performed on either 1.5 Tesla or 3 Tesla MRI systems were also required at both imaging time points. Using these screening criteria, a total of 91 patients were selected.

#### *Disease Progression Assessment*

RANO criteria, including changes in T1-weighted gadolinium-enhancing lesions, as well as non-enhancing T2/FLAIR areas of abnormality, were used to assess disease progression.<sup>53</sup> PFS and OS were calculated with respect to the date of bevacizumab therapy initiation.

#### *Enhancing and Nonenhancing Lesion Volume Segmentation*

As previously described for the volumetric assessment of Study 2, whole brain post-contrast T1 weighted and T2/FLAIR images from MRI obtained at baseline and after initiating bevacizumab were used for tumor volume segmentation using 3D Slicer Software (version 4.1, Boston, MA)<sup>81,82</sup> by an investigator who was blinded to clinical outcomes. In this study, the T2/FLAIR abnormality volume that overlapped with the enhancing lesion volume was subtracted so that the final T2/FLAIR volume only included the non-enhancing component of abnormality.

#### *Coregistration with ADC maps*

Prior to mapping the segmented enhancing and nonenhancing lesion volumes onto the ADC volume, the T1+C and T2/FLAIR whole brain volumes were spatially co-registered to the

whole brain ADC volume using linear registration with affine transformation (12 degrees of freedom). The ADC values of individual pixels within the T1+C and T2/FLAIR volumes were then plotted into histograms. The schematic outline of volume segmentation and co-registration with ADC map is illustrated in Figure 6.

#### *Curve-fit analysis of Histogram of ADC volumes*

Curve-fitting and area integrations were performed using multiple Gaussian functions with peak center assignments based on previously reported ADC values of WHO grade III astrocytoma ( $1245 \pm 153 \times 10^{-6} \text{mm}^2/\text{sec}$ ) and glioblastoma ( $1079 \pm 154 \times 10^{-6} \text{mm}^2/\text{sec}$ ),<sup>64</sup> as well as peritumoral edema ( $1420$  to  $1825 \times 10^{-6} \text{mm}^2/\text{sec}$ ).<sup>83,84</sup> Due to overlapping ADC values between WHO grade III astrocytoma and glioblastoma, we defined pixels with ADC values lower than  $1050 \times 10^{-6} \text{mm}^2/\text{sec}$  as the component of glioblastoma distinct from WHO grade III astrocytoma and assigned one Gaussian peak ( $\text{ADC}_L$ ) to fit this lowest ADC region in the ADC histogram. For the histogram component with ADC values greater than  $1050 \times 10^{-6} \text{mm}^2/\text{sec}$ , we used two separate Gaussian peaks ( $\text{ADC}_{M1}$  and  $\text{ADC}_{M2}$ ) using fixed peak centers at  $1150 \times 10^{-6} \text{mm}^2/\text{sec}$  and  $1350 \times 10^{-6} \text{mm}^2/\text{sec}$  because the fitting appeared more reproducible compared to single broad peak assignment. We also assigned a fourth peak,  $\text{ADC}_H$ , with a peak center at  $1550 \times 10^{-6} \text{mm}^2/\text{sec}$  to account for peritumoral edema and regions of radiation leukoencephalopathy. In summary, the peak parameters were specified as the following:  $\text{ADC}_L$  (center  $< 1050 \times 10^{-6} \text{mm}^2/\text{sec}$ ),  $\text{ADC}_M$  (M1: center =  $1150 \times 10^{-6} \text{mm}^2/\text{sec}$ , width = 140, M2: center =  $1350 \times 10^{-6} \text{mm}^2/\text{sec}$ , width = 140),  $\text{ADC}_H$  (center =  $1550 \times 10^{-6} \text{mm}^2/\text{sec}$ ) (Figure 7). The convergence criteria were determined by Levenberg–Marquardt algorithm. The peaks were area-integrated and expressed as ratios, including % $\text{ADC}_L$  ( $\text{ADC}_L$  area / total Area), % $\text{ADC}_H$  ( $\text{ADC}_H$  / total

area), and  $ADC_L / ADC_M$  ( $ADC_L$  area /  $ADC_M$  area). All curve fitting was done using Fityk (version 0.9.8, <http://fityk.nieto.pl>).

### *Statistical analysis*

The primary outcome measures were PFS and OS. The Kaplan-Meier method was used to provide median point estimates and time specific rates. The Cox proportional hazards model was used in uni-variable and multi-variable settings to identify ADC imaging markers significantly associated with PFS and OS. Following analysis of clinical and ADC parameters, the sample was dichotomized by median values for each ADC parameter. Appropriate subanalyses were planned to account for significant clinical variables found in the initial analyses. All of the statistical analyses were performed using STATA, version 12.0 (College Station, TX).

## **Results**

### *Patient characteristics*

The patient characteristics of this single institution database of patients have already been discussed in the Results section of Study 2. The patient characteristics and baseline clinical variables with respect to PFS and OS are summarized in Table 3. Among clinical variables, number of recurrences and change in steroid dose from baseline to post-treatment scan were associated with PFS and OS.

### *Analysis of Imaging Parameters as Continuous Variables Adjusted for Clinical Parameters*

Each ADC parameter was first evaluated individually with clinical variables including age, gender, KPS, number of recurrences, baseline steroid dose, change in steroid dose, treatment



regimen (bevacizumab monotherapy vs. concurrent chemotherapy), and re-resection before treatment in a Cox proportional hazards model (Table 6). Among the ADC variables, baseline %ADC<sub>H</sub> within T1+C, ADC<sub>L</sub>/ADC<sub>M</sub> within T2/FLAIR and post-treatment %ADC<sub>L</sub> within T1+C and ADC<sub>L</sub>/ADC<sub>M</sub> within T2/FLAIR were associated with both OS and PFS. None of the variables representing change in ADC parameters before and after treatment predicted PFS or OS.

#### *Multivariable analysis of dichotomized ADC parameters*

Subsequently, the four continuous ADC parameters with significant associations with OS (as well as PFS), specifically baseline %ADC<sub>H</sub> within T1+C, baseline ADC<sub>L</sub>/ADC<sub>M</sub> within T2/FLAIR, post-treatment %ADC<sub>L</sub> within T1+C, and post-treatment ADC<sub>L</sub>/ADC<sub>M</sub> within T2/FLAIR, were dichotomized by median values and were then included in multivariable models along with the same clinical variables and T1+C volume at baseline (Table 7). Baseline volume parameters (enhancing tumor volume, %ADC<sub>H</sub> within T1+C < or > 25%, ADC<sub>L</sub>/ADC<sub>M</sub> within T2/FLAIR <= or > 0.64) were significantly associated with both OS and PFS. The Kaplan-Meier survival plots using %ADC<sub>H</sub> within T1+C, ADC<sub>L</sub>/ADC<sub>M</sub> within T2/FLAIR are shown in Figure 8A-D. To account for both ADC parameters, we introduced a combined ADC factor, defined as %ADC<sub>H</sub> within T1+C divided by ADC<sub>L</sub>/ADC<sub>M</sub> within T2/FLAIR. A cut-off of 0.8 (n= 35 for factor > 0.8 and n= 56 for factor <= 0.8) was used for dichotomization of this factor after achieving the lowest hazard ratio for OS (Uni-variable: HR= 0.43, p =0.002; multi-variable adjusted for clinical variables: HR= 0.17, p < 0.0001) (Figure 8E and 8F).

#### *Stratifications of patient survival using baseline enhancing tumor volume and combined baseline ADC Factor*

To examine whether the combined baseline ADC factor can improve stratification of patient survival when assessed together with baseline volume of contrast enhancement, we first dichotomized the total patient group using the baseline T1+C volume of 20cc, followed by a second stratification using the combined baseline ADC factor with the aforementioned threshold of 0.8. Kaplan-Meier estimates of overall survival were calculated for each of these groups (Figure 9). Based on the Cox proportional hazards model, the ADC factor was still significantly associated with OS in both subgroups, among patients with baseline enhancing volume > 20cc (HR= 0.40,  $p = 0.017$ ) and patients with baseline enhancing tumor  $\leq 20$ cc (HR= 0.45,  $p = 0.039$ ). Among these 4 groups, patients with smaller pretreatment volume, defined as  $\leq 20$ cc, with a combined baseline ADC factor of > 0.8, had the longest median overall survival of 67.1 weeks. Patients with ADC factors of  $\leq 0.8$  and baseline volume > 20cc had the shortest median overall survival of 24.1 weeks.

#### *Subgroup Analysis: Recurrences*

Although the recurrences were not associated with overall survival in our multivariable analysis, it is important to test whether the ability of ADC parameters to stratify patient survival is affected by this variable. We examined ADC parameters in subsets of patients at first, second, and third recurrence (Table 8). For patients at first recurrence ( $n=47$ ), both baseline %ADC<sub>H</sub> within T1+C and ADC<sub>L</sub>/ADC<sub>M</sub> within T2/FLAIR were able to stratify the sample for survival using the previously listed median values for each parameter ( $p = 0.037$  and  $p < 0.0001$ , respectively). For patients at second and third recurrence with relative smaller sample size ( $n=29$  and 15, respectively), neither ADC parameter was able to stratify patients for survival.

#### *Subgroup Analysis: Bevacizumab monotherapy vs. bevacizumab with concurrent therapies*

In order to account for effects of heterogeneous concurrent therapies with bevacizumab, we examined the relationship of ADC parameters among the subset of patients who received only bevacizumab monotherapy and those who received concurrent chemotherapy with Kaplan-Meier estimates. For patients with bevacizumab monotherapy ( $n = 47$ ), both baseline  $\%ADC_H$  within T1+C and  $ADC_L/ADC_M$  within T2/FLAIR were able to stratify the sample for survival ( $p=0.07$ ,  $p=0.013$  respectively). For patients with concurrent therapy in combination with bevacizumab ( $n=44$ ), neither ADC parameter was able to stratify the sample for survival ( $p=0.56$  and  $p=0.076$ , respectively).

## **Discussion**

We analyzed the histogram of ADC maps from baseline and early post-treatment MRI exams in patients with recurrent glioblastoma treated with bevacizumab. In addition to evaluating enhancing tumor volume on T1-weighted images, we also characterized nonenhancing volumes based on T2/FLAIR images and hypothesized that the ADC histogram analysis of the nonenhancing component of tumors can serve as an independent imaging biomarker. Multi-component curve-fitting of T2/FLAIR abnormality at baseline MRI revealed a significant association between patient survival and the ratio of the  $ADC_L$  peak to the  $ADC_M$  peak. These ADC values have been observed in glioblastoma and WHO grade III astrocytoma, with the lower peak ( $ADC_L$ ) reported in glioblastoma but not in WHO grade III astrocytoma.<sup>64</sup> While the exact mechanism of our results is unclear, it is possible that patients with a greater  $ADC_L / ADC_M$  component had a greater proportion of volume within the non-enhancing T2/FLAIR abnormality behaving more aggressively or being more resistant to anti-angiogenic therapy. Since the introduction of anti-angiogenic therapy, growth of non-enhancing infiltrative tumor has been recognized as a type of tumor,<sup>45</sup> and diffusion imaging has been shown to be able

to detect nonenhancing tumor progression.<sup>98</sup> Our results provide evidence that the proportion of nonenhancing tumor likely contributing to unfavorable survival outcome can be assessed prior to treatment by analyzing ADC histograms within the T2/FLAIR abnormality. The ability to assess this feature prior to initiating therapy makes it a particularly noteworthy finding.

We also attempted to confirm the clinical significance of ADC histogram analysis within enhancing tumor on the baseline MRI as has been previously demonstrated in several studies. Rather than using a two-component curve-fitting analysis as done in prior studies,<sup>67–69,78,79</sup> we used a four-component curve-fitting algorithm. The inclusion of two additional peaks improved the accuracy and reproducibility of histogram fitting based on both visual inspection and residual function, and allowed for the identification of sub-components with ADC values corresponding to those observed in different histological grades of glioma. Using this peak fitting algorithm, we observed that a greater percentage of the highest ADC peak area (%ADC<sub>H</sub>) is associated with longer PFS and OS. This finding is concordant with prior two-component analysis of histogram that showed that a greater percentage of the lower ADC peak (or a smaller percentage of the higher ADC peak) in the bevacizumab treated group is predictive of shorter PFS.<sup>78</sup> In contrast to the analysis of the T2/FLAIR component, however, the ratio of ADC<sub>L</sub> to ADC<sub>M</sub> within T1+C was not associated with patient survival in our study (Table 6), indicating that the ratio between the ADC<sub>L</sub> and ADC<sub>M</sub> components of the enhancing volume is less important with respect to survival according to our results.

A closer inspection of the Kaplan-Meier survival curve stratified by ADC<sub>L</sub>/ADC<sub>M</sub> within T2/FLAIR revealed that the separation of the curves is larger during the latter part of treatment course (Figure 8C), while the separation of survival curves stratified by %ADC<sub>H</sub> within the enhancing tumor is noticeable even at an early time point following treatment initiation (Figure 8A). Quantitatively, the 75<sup>th</sup> percentile overall survival is 391 versus 695 days for ADC<sub>L</sub>/ADC<sub>M</sub>

(T2/FLAIR) larger or smaller than 0.64. For %ADC<sub>H</sub> (T1+C) the 75<sup>th</sup> percentile overall survival is 421 versus 568 days. Although speculative, these observations suggest that the subcomponents of nonenhancing tumor, while not necessarily helpful in identifying patients who progress very early following treatment, may identify patients who are long-term survivors following treatment. Further research will be required to investigate this hypothesis.

In order to account for both the enhancing and nonenhancing components of tumor, we proposed a combined ADC factor, dividing %ADC<sub>H</sub> (T1+C) by ADC<sub>L</sub>/ADC<sub>M</sub> (T2/FLAIR). Using 0.8 as cut-off to stratify patients into groups with favorable and unfavorable ADC characteristics, the median OS was 224 vs. 455 days ( $p < 0.002$ , HR=0.43) (Figure 8E). The ratio of median OS in patient groups stratified by the combined ADC factor is 2.03, larger than either %ADC<sub>H</sub> within T1+C volume (1.3) or ADC<sub>L</sub>/ADC<sub>M</sub> within T2/FLAIR (1.86) alone. Furthermore, we have shown in our volumetric analysis of conventional MRI that the absolute tumor volume is an imaging marker correlating with patient survival. Using the combined ADC factor, we further stratified patient subgroups with different pretreatment volumes ( $>$  or  $\leq$  20cc), resulting in significant differences in both OS and PFS for both volume groups (Figure 9). This represents a method to combine volumetric approach with ADC histogram to demonstrate that they are both individually helpful in stratifying patients.

There has now been a significant amount of research demonstrating the possible benefits of ADC histogram analysis of the baseline scan prior to initiating bevacizumab. In this single institution study, all patients received bevacizumab and there was no comparative arm. While ADC histogram analysis appears to be an imaging biomarker that can stratify for PFS and OS, our study was unable to determine whether this serves a predictor to bevacizumab therapy or if it is simply a prognostic marker independent of therapy. Ellingson et al. (2013) has recently demonstrated that the baseline ADC histogram analysis does not stratify recurrent glioblastoma

patients for PFS or OS in patients who are receiving systemic chemotherapy (and not bevacizumab).<sup>79</sup> If corroborated, this would indicate that ADC histogram analysis may have the potential to be used to identify patients who are most likely to achieve a durable benefit from bevacizumab. Given that most other imaging biomarkers that are being evaluated as predictors of response in bevacizumab involve early post-treatment imaging,<sup>73,74,100–102</sup> the use of ADC histogram analysis requires further investigation with respect to its ability to identify patients likely to receive a durable benefit from bevacizumab *prior to* treatment initiation.

Assessment of ADC histograms from early (3-6 week) post-treatment MRI may also provide useful prognostic information immediately following the first few doses of treatment. While several post-treatment ADC parameters appear to be associated with PFS and OS (Table 6), the associations were not significant in multi-variable analysis when assessed with pretreatment ADC parameters and clinical variables (Table 7). A number of possibilities can contribute to the component of low ADC lesions following anti-angiogenic therapy. For example, prior studies have shown that low ADC lesions can be seen following treatment and represent chronic hypoxia and atypical gelatinous necrotic tissue.<sup>98,103</sup> In addition, areas of prior edema can normalize, and the ADC value may approach that of normal white matter, which is also in the range overlapping with high grade glioma.<sup>65</sup> To better characterize the diffusion properties of tumor following treatment, further decomposition of the ADC<sub>L</sub> peak may be helpful. High b-value diffusion is a promising technique that potentially can better characterize the ADC<sub>L</sub> peak as evidenced by prior studies.<sup>104</sup> With the techniques used in this study, however, post-treatment ADC parameters did not appear to be as helpful as baseline imaging parameters.

The change of ADC parameters before and after treatment initiation also did not correlate with survival. ADC parameters were ratios of peak areas and there is often significant difference between volumes before and after treatment, and direct subtraction likely does not represent the

actual volume change of a particular sub-peak. Even if adjusted for volume, the location information is lost during analysis. The voxel-subtraction method of fDM, however, has the advantage of assessing change in ADC within all regions of tumor or adjacent brain, thus should be more accurate in reflecting treatment-related effect. Several prior studies have demonstrated the value of this method in correlating serial ADC change to survival.<sup>70,71</sup> It is worth noting that the parameters from histogram analysis of pretreatment MRI and fDM analysis of serial MR imaging can be complementary and used together in the future.

### *Study 3 Limitations*

First and foremost, as with the other parts of this project, the findings from our retrospective study need to be confirmed in future prospective trials. As previously alluded to, there was no control group that did not receive anti-angiogenic therapy, thus not allowing for confirming the prognostic or predictive nature of the ADC parameters with respect to patient survival outcome. While others have supported the notion that the pre-treatment parameters from ADC histogram analysis can serve as a predictor of response specific for bevacizumab therapy,<sup>79</sup> we were unable to investigate this. Nearly half of our patients received one or more concurrent chemotherapies in addition to bevacizumab, making it more difficult to draw conclusions regarding treatment-specific benefits of our approach. Nonetheless, subgroup analysis of patients who only received bevacizumab monotherapy indicated that the association of ADC parameters remains significant with respect to OS. Furthermore, the number of recurrences included in our patient selection criteria is not uniform, ranging between 1 and 3. Interestingly, the significant association of ADC parameters with patient survival was observed only in the group with one recurrence in our subanalyses, again suggesting that alterations of ADC

characteristics due to successive treatment regimens may confound histogram analysis that is based on previously established ADC values of untreated tumor.

As with Study 2, the distinction of pseudoprogression and early recurrence is important because its misclassification can affect outcome assessment. As already described, several measures were taken to minimize the possible impact of pseudoprogression.

While our multi-component approach to ADC histogram approach allowed for greater decomposition of enhancing and nonenhancing tumor, it is not clear if this is a better approach than the more commonly employed bi-component analysis. We utilized a four-component model because of better visual fit and improved residual function, but a comparative study would be needed to better evaluate this question. Furthermore, pathological correlates via prospective studies involving biopsy of areas corresponding to different peaks of ADC histograms would be the gold standard for validating our interpretation of our multi-component curve fitting technique.

#### *Future Direction for Prognostic/Predictive Imaging Biomarker Research*

The results from the volumetric and ADC histogram study add to a growing body of knowledge regarding imaging biomarkers in recurrent glioblastoma. Several barriers remain prior to implementation of these imaging biomarkers in everyday clinical use. First, there is a great degree of heterogeneity with respect to exact parameters used to acquire MRI imaging at centers around the country and world. The cutoff values presented in this project represent the results from a single institution experience that cannot be necessarily generalized to the broader glioblastoma population. Standardization of methods is logistically challenging but would represent an important step towards allowing for better evaluation of research and facilitating eventual acceptance of specific cutoff values used for volumetric and ADC parameters.



Furthermore, large multi-institutional, prospective studies are needed to verify the importance of volumetric segmentation and ADC histogram analysis in recurrent glioblastoma patients being initiated on bevacizumab. Hence, future research should be aimed at validating findings that have accumulated in support of volumetric analysis and ADC histogram analysis as imaging biomarkers in bevacizumab-treated recurrent glioblastoma patients.

With so many advanced imaging techniques in development, each one has become increasingly recognized to be valuable in terms of characterizing different pathophysiological aspects of tumor and treatment changes in the setting of inhibitors of angiogenesis. While individually helpful, the combination of these techniques represent a promising way of creating powerful imaging biomarkers and metrics that may better predict and assess response to therapy. In our ADC histogram analysis study, we demonstrated that absolute volume and baseline ADC parameters can be utilized together to allow for stratification of patient groups by survival (Figure 9). More sophisticated means of utilizing the information from different techniques can offer more powerful stratifications. Multiparametric approaches have been utilized in high-grade gliomas for other purposes (e.g. differentiating recurrence from radiation necrosis),<sup>116</sup> and future research should explore their ability to be generate imaging biomarkers in recurrent glioblastoma patients being initiated on bevacizumab.

### *Study 3 Conclusion*

We assessed several ADC parameters derived from histogram analysis of contrast-enhancing and T2/FLAIR nonenhancing areas of tumor in patients with recurrent glioblastoma being initiated on bevacizumab therapy. Our analysis indicates that ADC histogram analysis within both enhancing and nonenhancing components of tumor may be used in combination to stratify patients with respect to PFS and OS.

## Summary

The overarching theme of the project was to examine the utilization of MRI in recurrent glioblastoma with respect to response assessment and as imaging biomarkers that may have a prognostic or predictive role. In Study 1, our goal was to validate the RANO criteria for response assessment in recurrent glioblastoma by performing a comparative analysis with the Macdonald criteria. In the second part of the project, our goal was to evaluate imaging biomarkers derived from (a) volumetric analysis of conventional MRI (Study 2) and (b) histogram analysis of DWI-MR of both enhancing and nonenhancing tumor (Study 3) in their ability to stratify patients for PFS and OS.

Taken together, we believe that the results of our project provide important advances in the imaging of recurrent glioblastoma. Given the need for better therapeutics for recurrent glioblastoma because of its dismal prognosis, clinical trials are essential for discovering improved therapeutics, and response assessment represents a critical aspect of treatment evaluation. Based upon improvements relative to the Macdonald criteria, the RANO criteria provides several advantages in better determining response and progression for therapies, particularly in the setting of new anti-angiogenic agents. By using outcomes and imaging from a phase II clinical trial of bevacizumab in recurrent glioblastoma, we have demonstrated that the RANO criteria improves determination of progression through its consideration for nonenhancing tumor apparent on T2/FLAIR imaging. As the imaging endpoints determined by the RANO criteria correlate with subsequent survival, our results support the use of RANO in clinical trials of high grade gliomas.

Despite the progress represented by the RANO criteria, the advent of advanced imaging techniques has the potential of better identifying patients likely to benefit from certain therapies and to serve as biomarkers with prognostic or predictive significance. A volumetric assessment of enhancing tumor prior to and early in treatment appears to be beneficial in both identifying patients more likely to achieve a durable response with bevacizumab. Similarly, our work supports the potential use of ADC histogram analysis of both enhancing and nonenhancing components of tumor as a predictive imaging biomarker to identify patients who are likely to benefit from bevacizumab prior to initiating therapy. While our retrospective studies contribute to the literature, prospective research will be required to validate these approaches, as well as other approaches utilizing other advanced imaging techniques. With standardization and validation, these advanced imaging techniques can ultimately be incorporated into everyday clinical use. Until these advanced techniques are better established, conventional MRI with two-dimensional measurements as described by the RANO criteria remains the primary means of response assessment in bevacizumab-treated recurrent glioblastoma patients. Nonetheless, the continued refinement and advancement of advanced techniques will be crucial in the path towards developing reliable imaging biomarkers that can be ultimately utilized to improve patient outcomes.

## Acknowledgements

While a large body of work is presented as a part of this thesis, the presented research has required the work and input of many people. I would like to especially thank Raymond Y. Huang, MD, PhD and Patrick Y. Wen, MD for their tremendous mentorship. This project would not be possible without their tireless support and guidance.

As of the submission of this thesis, each component of this project has been written up with the purpose of publication. The RANO validation study is a multi-institutional effort that includes collaborators from Mayo Clinic (statistics) and UCLA (variability). The manuscript for this study will be submitted in the near future, and I will be the second author. As first author, Dr. Huang's efforts are particularly notable in this study because he completed measurements and response/progression determination on each patient for this study.

The volumetric study has been published in *Cancer* in October 2013, and I am a co-first author with Dr. Huang on this publication (reference 88). The ADC histogram study has been accepted for publication in the *Journal of Neuro-Oncology*, and I am the first author for this with Drs. Huang and Wen as the senior authors.

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## Figures

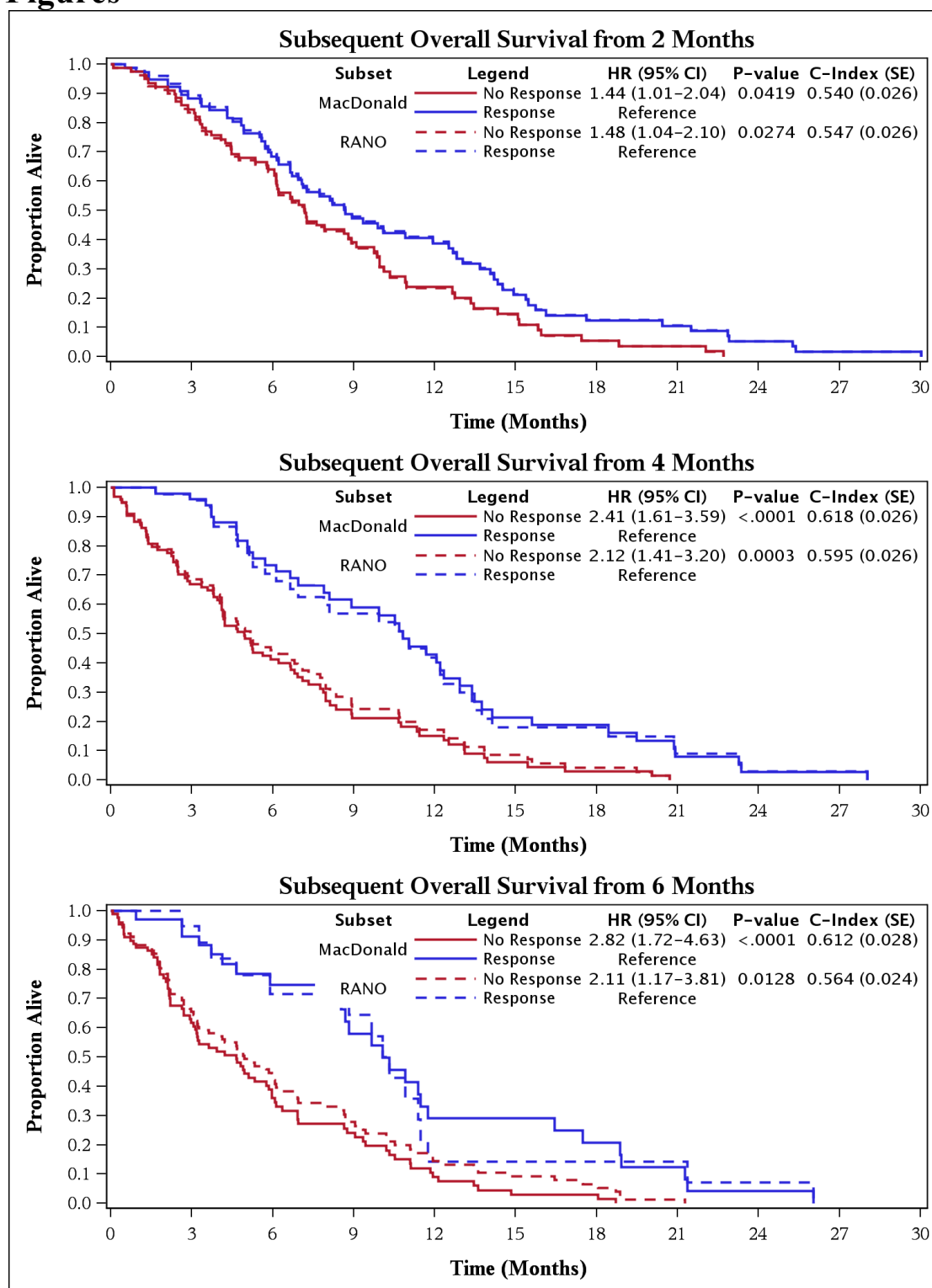


Figure 1: Kaplan-Meier curves of subsequent survival by response in landmark analysis performed at 2, 4 and 6 months.

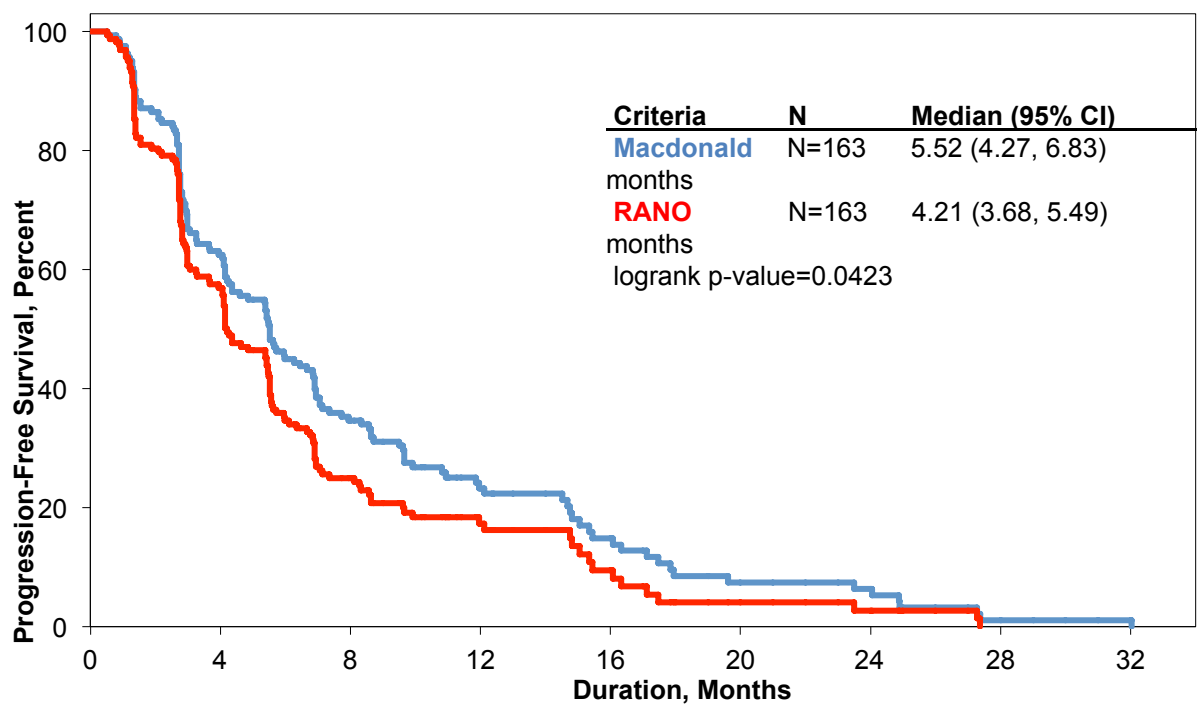


Figure 2: Kaplan-Meier estimates of PFS as determined by RANO and Macdonald response assessment criteria. By log-rank testing, there was a small but significant difference in PFS ( $p=0.0423$ ).



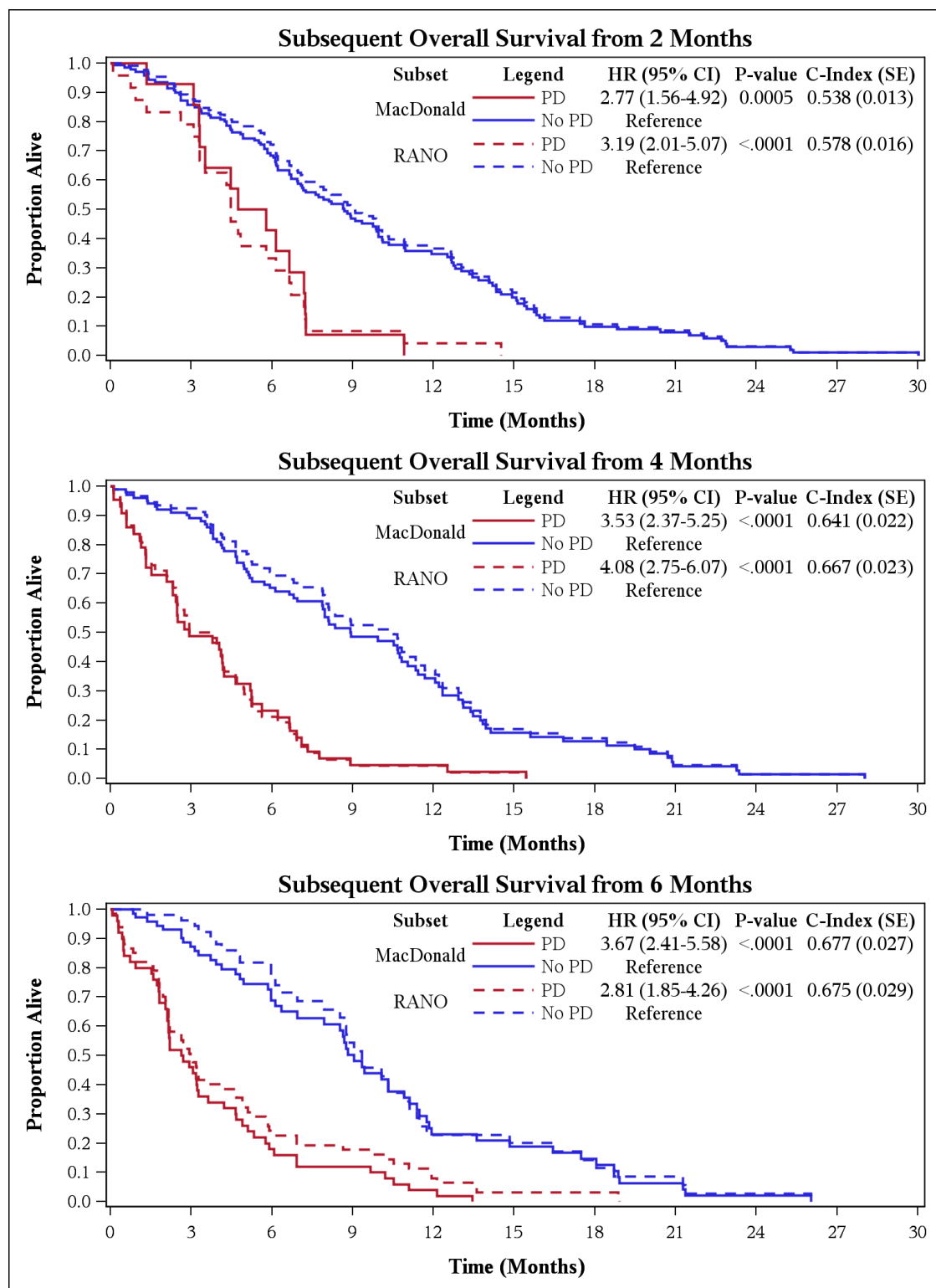


Figure 3: Kaplan-Meier curves of subsequent survival by progression in landmark analysis performed at 2, 4 and 6 months.

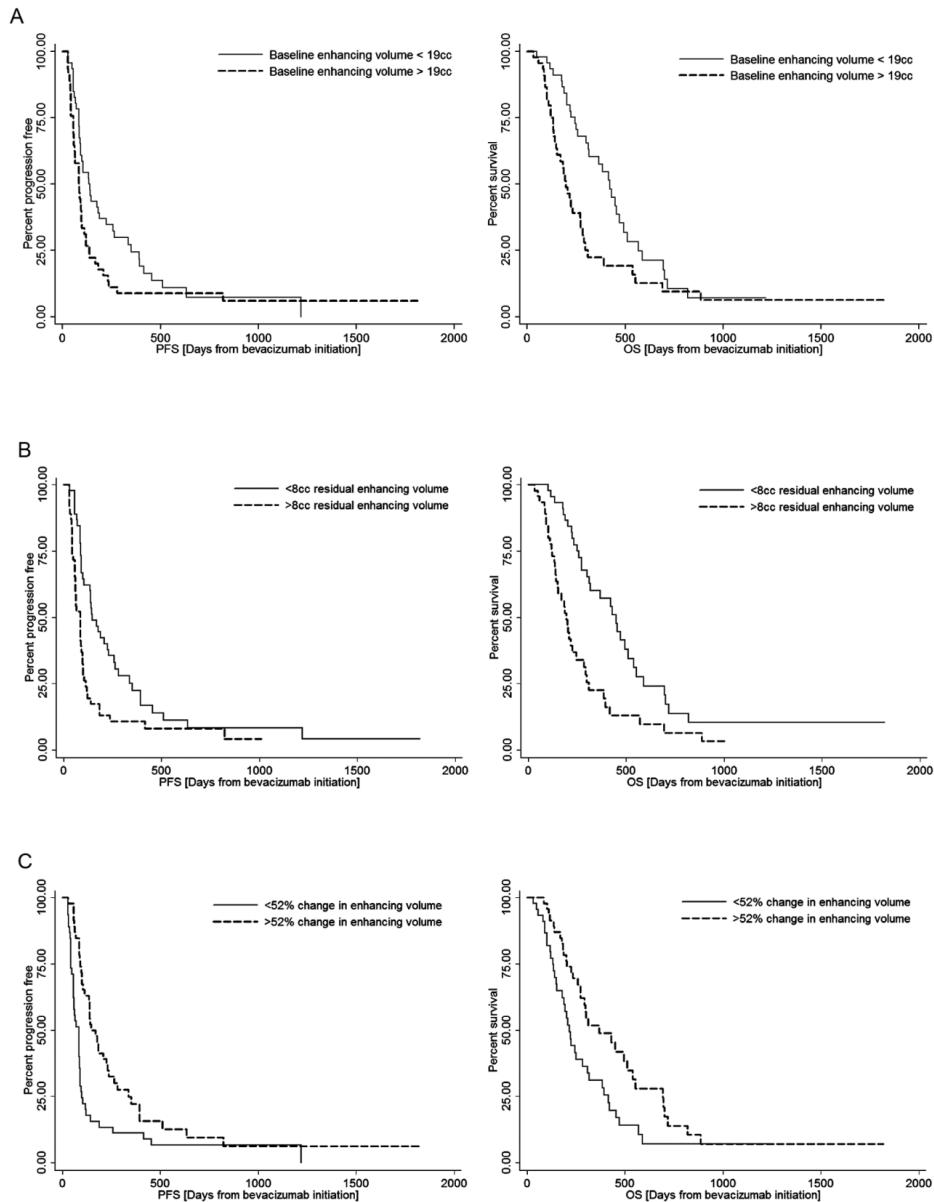


Figure 4\*: Volume parameters of enhancing lesion. Kaplan-Meier estimates of overall survival (left column) and progression-free survival (right column) for different T1+C volume parameters. The patient sample was divided into two groups by dichotomizing by the median value for (A) baseline T1+C volume, (B) post-treatment T1+C volume, and (C) the percentage change of T1+C volume between those two scans.

\* Huang RY, Rahman R, Hamdan A, et al. Recurrent glioblastoma: Volumetric assessment and stratification of patient survival with early posttreatment magnetic resonance imaging in patients treated with bevacizumab. *Cancer*. 2013. doi:10.1002/cncr.28210.

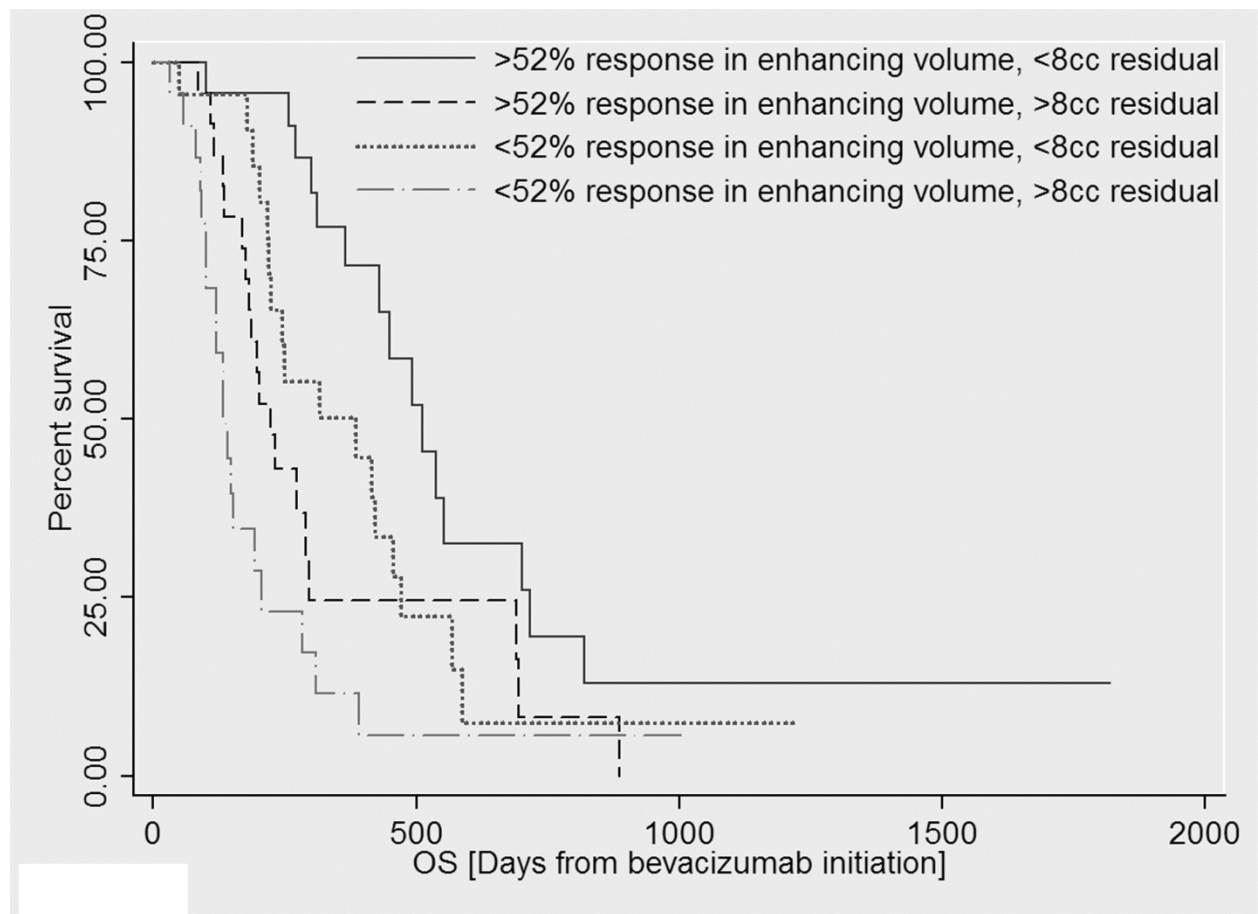


Figure 5\*: Combined stratifications using percentage volume change and post-treatment tumor volume.

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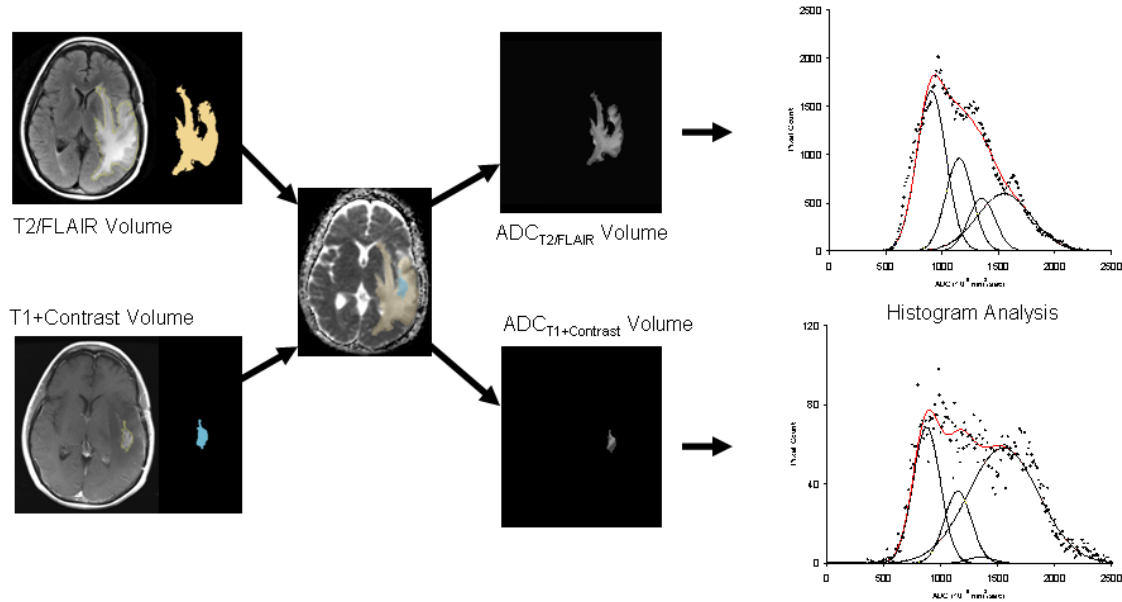


Figure 6<sup>#</sup>: Overview of tumor volume segmentation of T1+Contrast and nonenhancing T2/FLAIR hyperintensity. Nonenhancing T2/FLAIR volume was generated by subtracting the T1+Contrast volume from the total volume of T2/FLAIR abnormality. After co-registration, the T1+Contrast Volume and T2/FLAIR volume were mapped onto the ADC maps. The ADC values of individual pixels within the T1+C and T2/FLAIR volumes were then plotted into histograms.

<sup>#</sup> Rahman R, Hamdan A, Zweifler R, Jiang H, Norden AD, Reardon DA, Mukundun S, Wen PY, Huang RY. ADC Histogram Analysis of Apparent Diffusion Coefficient within Enhancing and Nonenhancing Tumor Volumes in Recurrent Glioblastoma Patients Treated with Bevacizumab. Accepted for publication in the *Journal of Neuro-Oncology*.

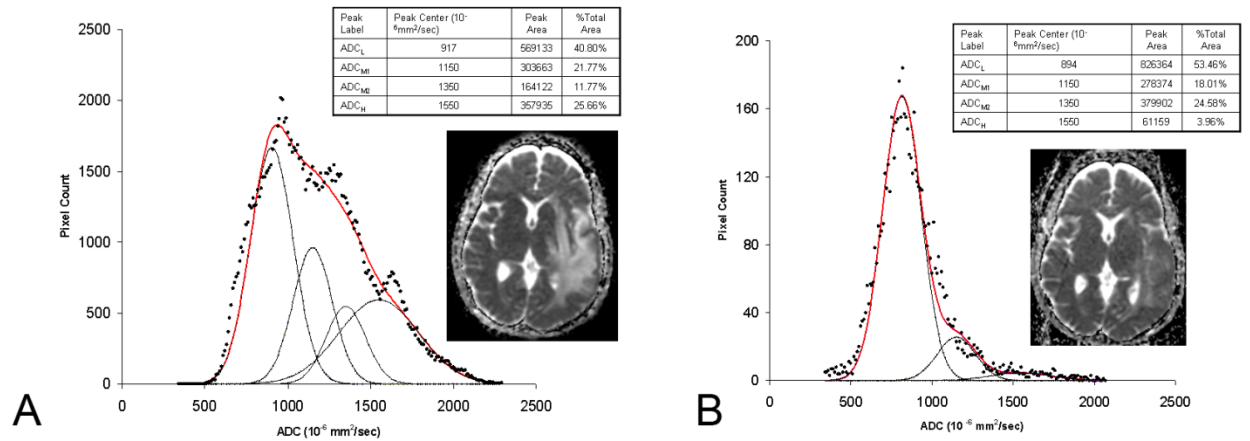


Figure 7: Curve-fitting and area integrations were performed using peak center assignments based on previous reported ADC values. The peak parameters are specified as the following: ADC<sub>L</sub> (center < 1050 x 10<sup>-6</sup> mm<sup>2</sup>/sec), ADC<sub>M</sub> (M1: center= 1150 x 10<sup>-6</sup> mm<sup>2</sup>/sec, width= 140 and M2: center=1350 x 10<sup>-6</sup> mm<sup>2</sup>/sec, width =140), ADC<sub>H</sub> (center = 1550 x 10<sup>-6</sup> mm<sup>2</sup>/sec). The peaks were area-integrated and expressed as ratios, including %ADC<sub>L</sub> (=ADC<sub>L</sub> area / total area), %ADC<sub>H</sub> (=ADC<sub>H</sub> / total area), and ADC<sub>L</sub> / ADC<sub>M</sub> (=ADC<sub>L</sub> area / ADC<sub>M</sub> area).

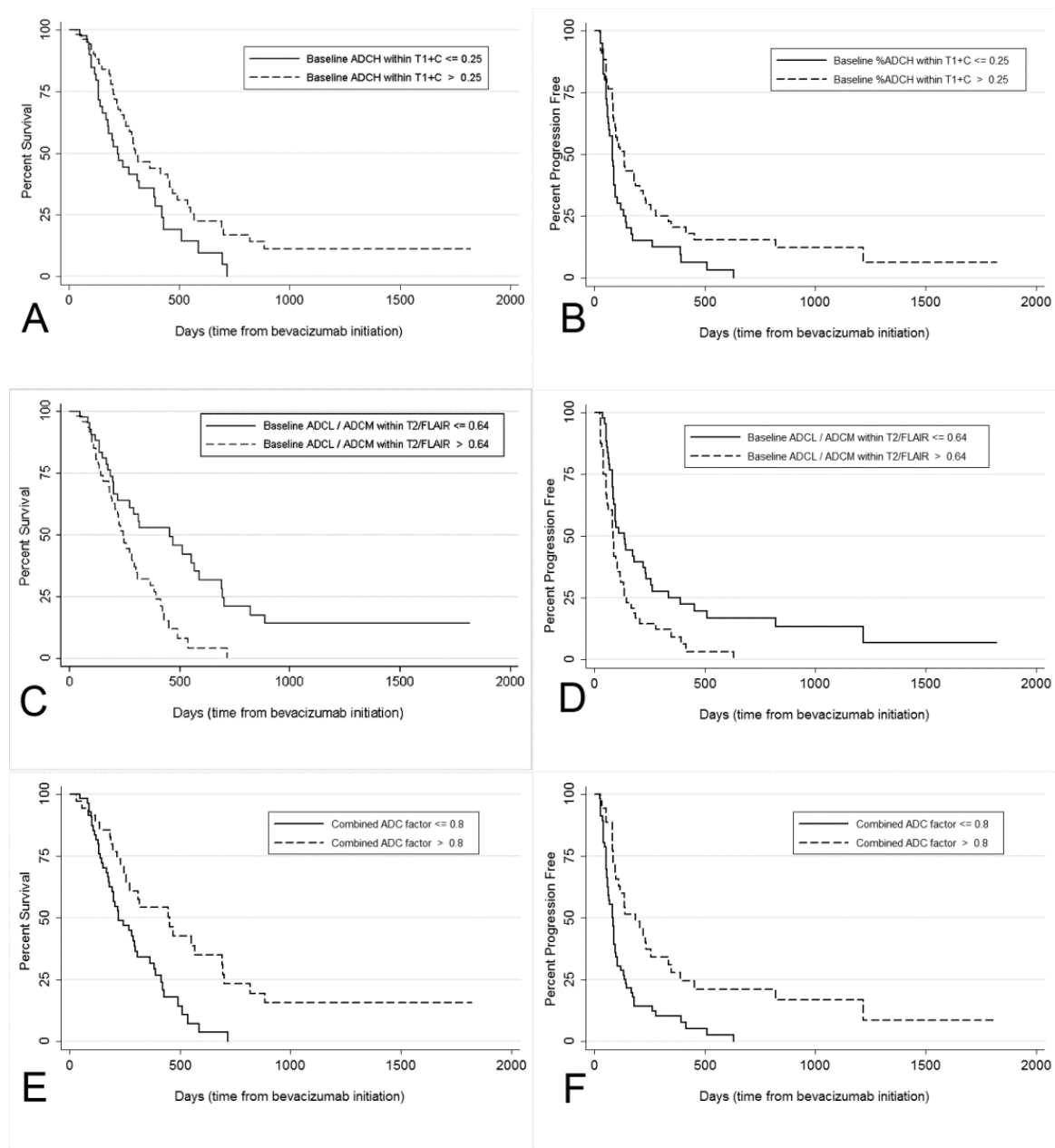


Figure 8<sup>#</sup>: Baseline ADC parameters. Kaplan-Meier estimates of overall survival (left column) and progression-free survival (right column) were calculated for the different ADC parameters. The patient sample was dichotomized by median value for (A) baseline  $\%ADC_H$  within T1+C, (B) baseline  $ADC_L / ADC_M$  within T2/FLAIR. In combining these two baseline ADC parameters, the patient sample was divided into 2 groups by dichotomizing by the optimized value of 0.8 for the (C) combined ADC factor.

<sup>#</sup> Rahman R, Hamdan A, Zweifler R, Jiang H, Norden AD, Reardon DA, Mukundun S, Wen PY, Huang RY. ADC Histogram Analysis of Apparent Diffusion Coefficient within Enhancing and Nonenhancing Tumor Volumes in Recurrent Glioblastoma Patients Treated with Bevacizumab. Accepted for publication in the *Journal of Neuro-Oncology*.

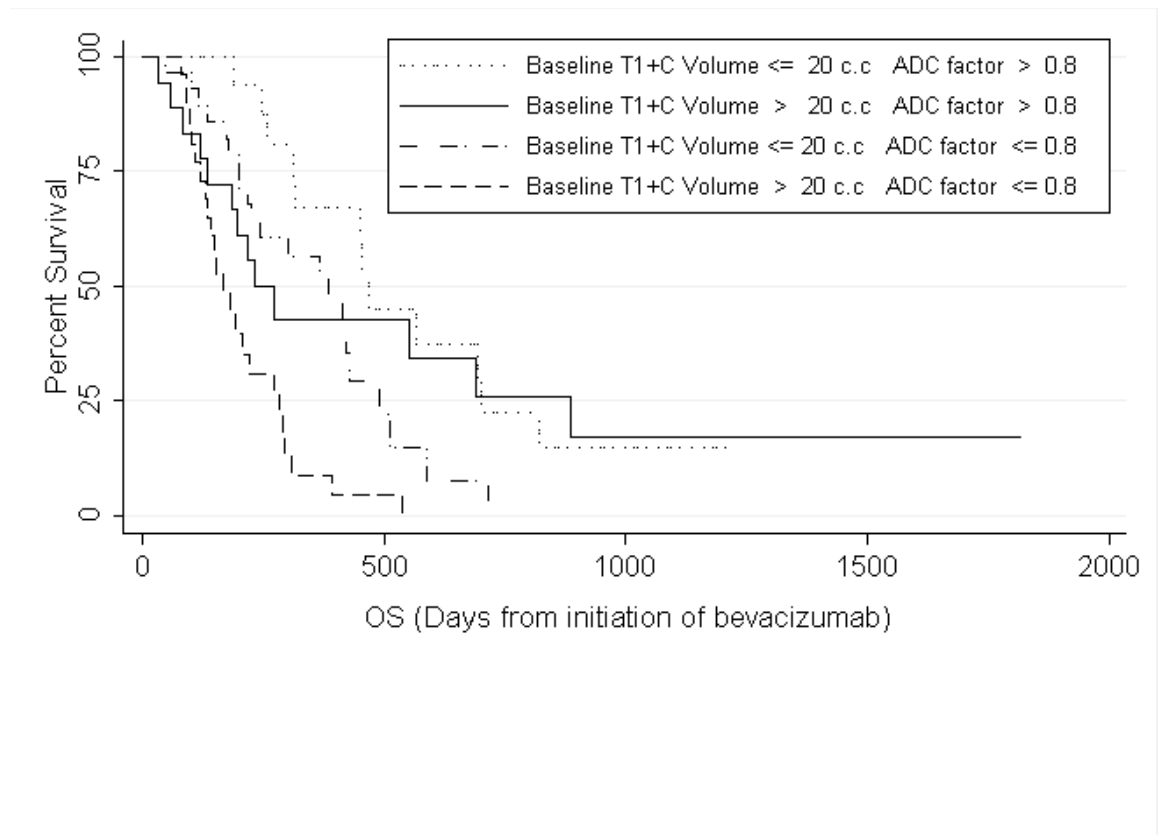


Figure 9: Combined stratification of the patient sample using the baseline enhancing tumor volume and combined ADC factor.

## Tables

Table 1: Patient characteristics from patients in the phase II BRAIN trial

Characteristic	% of Patients by Treatment Group	
	Bevacizumab alone	Bevacizumab and irinotecan
<i>Sex</i>		
Male	68.2	69.5
Female	31.8	30.5
<i>Relapse</i>		
First	81.2	80.5
Second	18.8	19.5
<i>Initial surgery</i>		
Partial resection	49.4	53.7
Complete resection	42.4	37.8
Biopsy only	8.2	8.5
<i>Enrollment relative to radiation</i>		
< 3 months from completion of radiotherapy	8.3	12.7
≥ 3 months from completion of radiotherapy	20.2	10.1



Table 2: Spearman correlation coefficients correlating TTP

	Other readers Macdonald PFS	Other readers RANO PFS	All readers RANO PFS
-Primary reader Macdonald PFS	0.688 <sup>a</sup>		
-Primary reader RANO PFS		0.692 <sup>a</sup>	
-All readers Macdonald PFS			0.781 <sup>b</sup>

<sup>a</sup>p < 0.0001 (n=115)

<sup>b</sup>p<0.0001 (n=278)

Table 3\*: Patient characteristics of institutional database

	OS		Median OS (weeks)	PFS		Median PFS (weeks)
	Hazard Ratio	P-value		Hazard Ratio	P-value	
Age (mean 56.3, range 23-83)	0.90	0.677	38.7	0.67	0.117	12.3
<65 (n=69)			44.6			20.0
>65 (n=22)						
Gender	1.36	0.210		0.89	0.614	
M (n=51)			21.1			13.7
F (n=40)			28.7			13.0
Number of recurrences	1.33	0.036		1.54	0.001	
1 <sup>st</sup> recurrence (n=47)			52.3			20.3
2 <sup>nd</sup> recurrence (n=29)			32.0			8.6
3 <sup>rd</sup> recurrence (n=11)			28.3			12.0
4 <sup>th</sup> recurrence (n=4)			35.6			11.9
Steroids at baseline (yes or no)	0.85	0.500	39.0	0.68	0.089	17.0
Steroids used (n=51)			41.4			12.7
Steroids not used (n=40)						
Change in steroid dose (from baseline to post-treatment)	0.92	<0.001	21.1	2.77	0.001	8.0
Increase (n=16)			45.1			14.9
Stable or decrease (n=75)						
Bevacizumab treatment regimen	1.37	0.188	44.6	1.15	0.542	14.9
Monotherapy (n=47)			38.7			12.7
With concurrent chemotherapy (n=44)						
Multifocal disease	1.04	0.912		0.78	0.467	
Yes (n=12)			38.7			19.4
No (n=79)			41.4			13.0
Time to bevacizumab initiation	1.00	0.690		1.00	0.061	

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Table 4\*: Volumetric parameters as continuous variables adjusted for clinical variables

	OS		PFS	
	Hazard Ratio <sup>a</sup>	p-value <sup>a</sup>	Hazard Ratio <sup>a</sup>	p-value <sup>a</sup>
<b>Volumetric Parameters</b>				
Baseline enhancing volume	1.02	<0.001	1.01	0.037
Post-treatment residual enhancing volume	1.03	<0.001	1.03	<0.001
Percent change in enhancing volume	0.56	0.002	0.48	0.001
Baseline FLAIR volume	1.00	0.15	1.00	0.57
Post-treatment FLAIR volume	1.01	0.025	1.01	0.018
Percent change in FLAIR volume	0.51	0.28	0.42	0.12
Baseline rNTR	0.98	0.16	0.99	0.29
Post-treatment rNTR	0.99	0.068	0.99	0.21

<sup>a</sup>Adjusted for age, KPS, number of recurrences, re-resection before treatment (yes or no), baseline steroid dose, change in steroids (yes or no) and treatment regimen (bevacizumab monotherapy vs. concurrent chemotherapy) within Cox proportional hazards model

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Table 5\*: Multivariable analysis using Cox proportional hazards model

	OS		PFS	
	Hazard Ratio	p-value	Hazard Ratio	p-value
Age (> or < 65)	2.30	0.088	0.58	0.21
Baseline KPS	0.97	0.18	0.99	0.94
Number of prior recurrences	1.54	0.062	1.65	<0.015
Re-resection prior to bevacizumab treatment	0.86	0.75	0.85	0.66
Baseline Steroid Dose	1.06	0.113	0.97	0.21
Steroid dose change (stable/decrease vs. increase)	1.29	0.663	2.4	0.065
Regimen (monotherapy vs. concurrent chemotherapy)	2.11	0.048	1.18	0.64
Baseline enhancing tumor volume	1.02	0.041	0.99	0.75
Post-treatment residual enhancing tumor volume	1.03	<0.001	1.04	<0.001
Post-treatment FLAIR	0.99	0.051	1.00	0.55

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Table 6<sup>#</sup>: ADC parameters as continuous variables adjusted for clinical variables

	OS		PFS	
	Hazard Ratio <sup>a</sup>	p-value <sup>a</sup>	Hazard Ratio <sup>a</sup>	p-value <sup>a</sup>
<b>Baseline ADC Parameters</b>				
%ADC <sub>L</sub> within T1+C	3.75	0.037*	2.87	0.062
%ADC <sub>L</sub> within T2/FLAIR	4.24	0.075	6.29	0.007*
%ADC <sub>H</sub> within T1+C	0.89	0.003*	0.169	0.006*
%ADC <sub>H</sub> within T2/FLAIR	0.69	0.69	0.329	0.22
ADC <sub>L</sub> / ADC <sub>M</sub> within T1+C	1.00	0.95	1.054	0.035
ADC <sub>L</sub> / ADC <sub>M</sub> within T2/FLAIR	1.23	0.014*	1.19	0.008*
<b>Post-Treatment ADC Parameters</b>				
%ADC <sub>L</sub> within T1+C	9.89	0.009*	7.032	0.010*
%ADC <sub>L</sub> within T2/FLAIR	4.27	0.093	5.919	0.022*
%ADC <sub>H</sub> within T1+C	0.19	0.085	1.731	0.029*
%ADC <sub>H</sub> within T2/FLAIR	0.65	0.75	0.444	0.032*
ADC <sub>L</sub> / ADC <sub>M</sub> within T1+C	1.01	0.71	1.016	0.41
ADC <sub>L</sub> / ADC <sub>M</sub> within T2/FLAIR	1.17	0.002*	1.037	0.028*
<b>Change before and after treatment</b>				
%ADC <sub>L</sub> within T1+C	1.060	0.93	2.26	0.13
%ADC <sub>L</sub> within T2/FLAIR	2.71	0.12	0.84	0.82

%ADC <sub>H</sub> within T1+C	1.82	0.53	0.83	0.76
%ADC <sub>H</sub> within T2/FLAIR	0.74	0.71	0.59	0.54
ADC <sub>L</sub> / ADC <sub>M</sub> within T1+C	0.99	0.75	0.99	0.94
ADC <sub>L</sub> / ADC <sub>M</sub> within T2/FLAIR	1.10	0.07	1.03	0.18

<sup>a</sup> Adjusted for age, KPS, number of recurrences, re-resection before treatment (yes or no), baseline steroid dose, change in steroids (yes or no) and treatment regimen (bevacizumab monotherapy vs. concurrent chemotherapy) within Cox proportional hazards model.

\* Significance level:  $p < 0.05$ .

# Rahman R, Hamdan A, Zweifler R, Jiang H, Norden AD, Reardon DA, Mukundun S, Wen PY, Huang RY. ADC Histogram Analysis of Apparent Diffusion Coefficient within Enhancing and Nonenhancing Tumor Volumes in Recurrent Glioblastoma Patients Treated with Bevacizumab. Accepted for publication in the *Journal of Neuro-Oncology*.

Table 7<sup>#</sup>: Multivariable analysis using Cox proportional hazards model

	OS		PFS	
	Hazard Ratio	p-value	Hazard Ratio	p-value
Age	1.067	0.002*	1.011	0.46
Baseline KPS	0.984	0.48	1.014	0.39
Number of prior recurrences	1.367	0.23	1.347	0.16
Re-resection prior to bevacizumab treatment	1.443	0.54	1.151	0.77
Baseline Steroid Dose	1.092	0.076	1.02	0.47
Steroid dose change (stable/decrease vs. increase)	0.092	0.47	0.86	0.04*
Regimen (monotherapy vs. concurrent chemotherapy)	1.418	0.42	0.911	0.795
Baseline enhancing tumor volume	9.361	<0.001*	2.556	0.029*
Baseline %ADC <sub>H</sub> within T1+C < or > 25%	0.341	0.012*	0.430	0.026*
Post-treatment %ADC <sub>L</sub> within T1+C <= or > 62%	1.90	0.19	1.962	0.071
Baseline ADC <sub>L</sub> /ADC <sub>M</sub> within T2/FLAIR <= or > 0.64	3.52	0.005*	2.822	0.02*
Post-treatment ADC <sub>L</sub> /ADC <sub>M</sub> within T2/FLAIR <= or > 1.85	1.06	0.89	0.503	0.098

\* Significance level: p<0.05.

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Table 8<sup>#</sup>: Median Overall Survival (days) stratified by baseline ADC parameters

	Baseline %ADC <sub>H</sub> within T1+C			Baseline ADC <sub>L</sub> /ADC <sub>M</sub> within T2/FLAIR		
	< 25%	> 25%	p-value*	<= 0.64	> 0.64	p-value*
Age						
<= 65 yr (n=69)	201	296	0.038	470	223	0.007
> 65 yr (n=22)	385	300	0.83	455	258	0.139
Number of prior recurrences						
1 (n=47)	220	449	0.037	552	258	< 0.0001
2 (n=29)	224	273	0.34	136	245	0.551
>=3 (n=15)	309	198	0.25	198	223	0.499
Treatment Regimen						
Bev Monotherapy (n=47)	141	300	0.007	455	245	0.013
Concurrent therapy (n=42)	385	290	0.56	511	249	0.075
Baseline enhancing tumor volume						
< 20 cm <sup>3</sup> (n=45)	385	455	0.09	511	336	0.045
> 20 cm <sup>3</sup> (n=46)	141	223	0.025	273	182	0.012

\* Significance level: p<0.05.

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